Municipal Library MAR 1 6 1932

VOLUME 2 MARCH, 1932 NUMBER 2

American Journal of Clinical Pathology

OFFICIAL PUBLICATION
THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

CONTENTS

Rhinosporidium seeberi in Nasal Polyp. The Fourth North American Case. GEORGE S. GRAHAM 73

Glycosuria and the Glycemic Tolerance Curve. A Review. EDGAR T. HERRMANN 87

Morphologic and Cultural Study of Staphylococci with Special Reference to Source. LUTHER THOMPSON 125

Modifications of Differential Stains with Special Reference to the Trichromic Stain of Cajal. RAMON CASTROVIEJO 135

Editorial 141

News and Notices 145

Book Reviews 151

PUBLISHED BI-MONTHLY BY THE WILLIAMS & WILKINS COMPANY
MOUNT ROYAL AND GUILFORD AVES., BALTIMORE, U. S. A.
Made in United States of America

American Journal of Clinical Pathology

EDITOR

T. B. MAGATH, Mayo Clinic, Rochester, Minnesota

ADVISORY EDITORIAL BOARD

C. S. BUTLER, U. S. Naval Medical School, Washington, D. C.

H. J. CORPER, National Jewish Hospital, Denver, Colo.

B. C. CROWELL, American College of Surgeons, Chicago, Ill.

HERBERT FOX, Pepper Laboratory of Clinical Medicine, University of Pennsylvania, Phila., Pa.

A. S. GIORDANO, 604 North Main Street, South Bend, Indiana.

F. W. HARTMAN, Henry Ford Hospital, Detroit, Mich.

R. A. KEILTY, U. S. Veterans' Bureau, Washington, D. C.

R. A. KILDUFFE, Atlantic City Hospital, Atlantic City, N. J.

J. A. KOLMER, Graduate School of Medicine, University of Pennsylvania, Philadelphia, Pa.

K. M. LYNCH, Medical College of the State of S. C., Charleston, S. C.

S. P. REIMANN, Lankenau Hospital, Phila., Pa.

A. H. SANFORD, Mayo Clinic, Rochester, Minnesota.

W. S. THOMAS, Clifton Springs Sanatorium, Clifton Springs, N. Y.

American Society of Clinical Pathologists

OFFICERS

President, H. J. CORPER, Denver, Colorado.

Vice-President, C. J. BUCHER, Philadelphia, Pennsylvania.

President-elect, W. M. SIMPSON, Dayton, Ohio.

Secretary-Treasurer, A. S. GIORDANO, South Bend, Indiana.

STANDING COMMITTEES

EXECUTIVE COMMITTEE

K. M. LYNCH, Chairman, Charleston, South Carolina.

F. W. HARTMAN Detroit, Michigan.

J. H. BLACK, Dallas, Texas.

F. E. SONDERN, New York City.

C. E. RODERICK, Battle Creek, Michigan.

A. G. FOORD, Pasadena, California.

BOARD OF CENSORS

F. H. LAMB, Chairman, Davenport, Iowa.

W. T. VAUGHAN, Richmond, Virginia.

W. E. KING, Detroit, Mich.

C. W. MAYNARD, Pueblo, Colorado.

NATHAN ROSENTHAL, New York City.

F. M. JOHNS, New Orleans, Louisiana.

BOARD OF REGISTRY

PHILIP HILLKOWITZ, Chairman, Denver, Colorado.

KANO IKEDA, St. Paul, Minnesota.

M. W. LYON, South Bend, Indiana.

R. W. HAMMACK, Los Angeles, California.

A. S. GIORDANO, South Bend, Indiana.

W. E. KING, Detroit, Mich.

A. YAGUDA, Newark, N. J.

Entered as second-class matter, January 7, 1931, at the Post-Office, at Baltimore, Md., under the act of March 3, 1879

Copyright 1933, The Williams & Wilkins Company

RHINOSPORIDIUM SEEBERI IN NASAL POLYP*

The Fourth North American Case†

GEORGE S. GRAHAM

Birmingham, Alabama

We owe to Ashworth[1] the first satisfactory description of the organism known since his work as *Rhinosporidium seeberi*. The organism was first recorded by Seeber[6] from Argentina in 1900 and was regarded as a protozoon allied to coccidium. Independent discovery of the organism was made in India whence the first case report was presented by O'Kinealy.[5] As in Seeber's case, the parasite occurred in a nasal polyp and the lesion was interpreted as a coccidial infection or local psorospermiosis. Minchin and Fantham[4] studied O'Kinealy's material and concluded that the organism possessed affinities with the Neosporidia and with the simpler Haplosporidia. They created for it the genus Rhinosporidium and appended the specific name *kinealyi*. This classification and name was generally adopted until Ashworth demonstrated Seeber's priority and emended the name to its present form. As a result of prolonged and painstaking study Ashworth was able to point out highly significant morphological inaccuracies in the earlier studies. Upon the basis of his own findings he reinterpreted and clarified the developmental cycle within the human tissues and concluded that the organism belongs to the lower fungi rather than to the sporozoa. He placed it therefore among the Phycomycetes and, in the absence of demonstrated mycelium, in the suborder Chytridineae.

No detailed statement of previous observations nor of the complete morphology will be attempted in the present report.

* Read before the Tenth Annual Convention of the American Society of Clinical Pathologists, Philadelphia, Pennsylvania, June 7–9, 1931.

† While this paper was in press another North American case was reported by W. L. Hanson in the Ann. Otology, Rhinol. and Laryng., **40**: 1013–1020, 1931.

At the best this could be no more than a condensation of the information contained in Ashworth's monograph while for the reader to whom that source is inaccessible the essential facts have been reviewed by Weller and Riker[7] in a recent report that is readily available to American readers. Since a brief summary may however be desirable in the interest of orientation it may be stated that recognized rhinosporidial infection is an uncommon lesion.

Some twenty-five or thirty cases have been reported from India and Ceylon. Three are known from Argentina, all noted by Seeber. An organism of apparently identical type was found in a nasal polyp removed from a horse in South Africa. At the time of Ashworth's publication (1922) only one case had been reported from North America. The most common site of infection is the nasal or the nasopharyngeal mucous membrane, but in occasional cases the lesion has occurred in the conjunctiva or the lachyrmal sac, the uvula, in one case in the external ear and in one upon the glans penis. Most of the subjects have been young persons. Thus of twenty-two cases in which the age is known, fourteen patients were less than twenty years of age, four were twenty-one to twenty-six years and four were older. The youngest patient was eight years old, the oldest fifty-five years. No case has yet been recorded in a female. Within the lesion the organism is found in great numbers and in all stages of development. The inflammatory tissue response results in polyp formation. Single or multiple lesions occur. The polyps grow slowly but in the nose may eventually produce marked obstruction, blocking the nasal passages and even projecting at the anterior nares or behind the uvula. In Seeber's case the tumor tissue removed weighed 20 grams. There is decided tendency toward recurrence after removal. Ashworth was able to follow his subject over a period of about four years during which time tumor tissue was removed on six occasions. The patient had already undergone operation six times during a preceding period of five or six years. Artificial culture has been attempted by several observers but with a single doubtful exception no growth has been obtained. Inoculation into a considerable

variety of animals including the monkey has likewise been without result.

The first case of this disease in North America was reported by Jonathan Wright[8] of New York. The subject was a farmer twenty years of age who had lived all his life near Memphis, Tennessee. He had undergone removal of recurrent and multiple nasal polyps several times over a period of five years. Sections of the tumor were submitted to Wright during the same year in which O'Kinealy was reporting his Indian case. On the basis of the early English descriptions, Wright identified the organism and reported his case four years later.

Lincoln and Gardner[3] reported the second American case in 1929. The subject was a forty year old man who had never been outside the United States and who had lived most of his life in the upper Mississippi valley region although there had been short periods of residence in Oklahoma and in Florida. His occupation is not stated. He had suffered a nasal discharge with occasional bleeding for about eight years when the nasal polyp was removed and the organism discovered in it.

The third case was reported by Weller and Riker.[7] The lesion was again a nasal polyp. The patient was a young man twenty-six years old, a native of Missouri. He had lived on a farm from the age of six to that of eighteen years and had never been outside the United States except for three days spent in Canada. The parents and five brothers and two sisters were living and well and none had suffered a similar condition. The nasal lesion was first noticed in 1926 shortly after the patient had received a nasal injury. A polyp was removed and there was no further complaint until after a second injury received early in 1928 when progressive obstruction developed in the nose on the side previously operated upon. A second operation was performed in May, 1929 and in the following month a third operation resulted in the removal of the polyp upon which the report was based.

THE PRESENT CASE

The patient was a negro boy twelve years of age. He was born on a farm in Georgia. When he was four years of age the family moved to New Castle, a

small coal mining village just outside Birmingham, Alabama, where the father has since worked as a miner. The boy had never been away from home except for a period of about six weeks spent on a farm in Montgomery, Alabama during the summer of 1922. He had an older and one younger brother and two sisters, all living and free of complaints, as were the parents. On October, 1929 the boy complained of an increasing nasal obstruction and was taken to the local physician, Dr. E. C. Payne, who referred him to the Norwood Clinic in Birmingham where Dr. H. S. Gherken removed a small polyp from the upper anterior surface of the cartilaginous septum on the right side just within the naris. The tumor was sent to the laboratory as a matter of routine and there received no particular attention until routine sections had come through and the organism was recognized. In July, 1930 the patient was seen and a recurrent polyp was found to be present at the previous site. It had excited no complaint and close questioning failed to reveal its probable duration. The patient did admit considerable mucous discharge from the affected side with occasional traces of blood. The polyp was now 1 cm. in diameter with broad base and dull red faintly lobulated surface scattered over which could be seen an occasional barely visible yellowish white dot.

The polyp was again removed with the snare and the area of attachment thoroughly cauterized. Before operation the nasal discharge was planted upon a variety of media including Saboraud's agar but the organism was not

---

FIG. 1. SPORES LYING FREE IN THE NASAL DISCHARGE

Their presence should render possible a pre-operative diagnosis of rhinosporidial infection. Dilute carbol-fuchsin stain. × 690.

FIG. 2. A TROPHIC FORM IN A MIDDLE STAGE OF DEVELOPMENT

A well defined pore is present in the plane of section. It is directed toward the epithelial surface. Eosin-methylene blue stain. × 160.

FIG. 3. RIPE SPORANGIUM DISCHARGING ITS SPORES INTO NASAL CAVITY

Note how the epithelium has disappeared over the bulging outer pole of the cyst. Eosin-methylene blue stain. × 160.

FIG. 4. A MATURED SPORANGIUM DISCHARGING ITS SPORES INTO THE SUBEPITHELIAL TISSUE

Early abscess formation. The space above the open pore contains free spores and leukocytes. Iron hematoxylin stain. × 80.

FIG. 5. SPORES LYING WITHIN A MATURED SPORANGIUM

A small sector of the marginal zone of undeveloped spores can be seen at the lower left hand margin. The section was stained by the eosin-methylene blue method and shows in each spore a sharply differentiated blue nucleus and red "spherules." This differentiation cannot be brought out in the black and white reproduction as sharply as in the original. × 970.

FIG. 1

FIG. 2

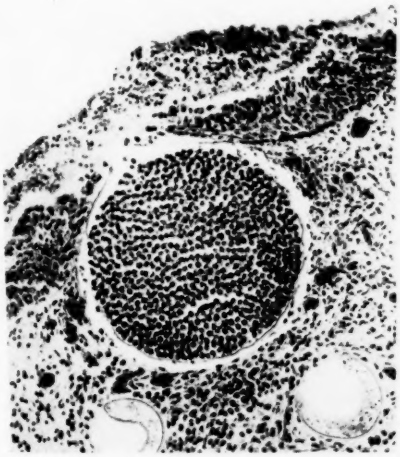

FIG. 3

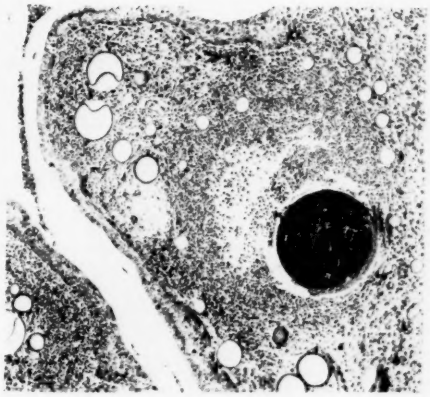

FIG. 4

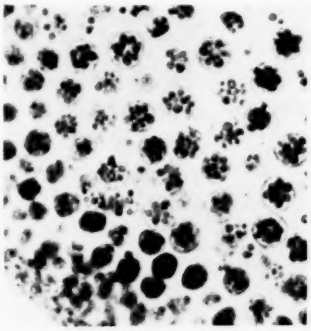

FIG. 5

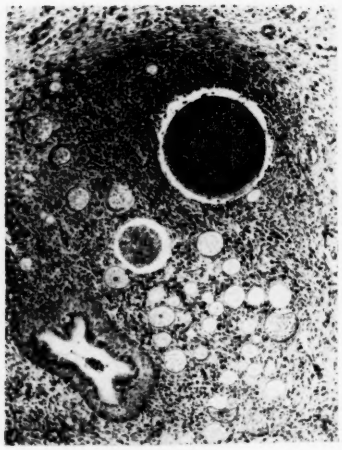

FIG. 6

FIG. 7

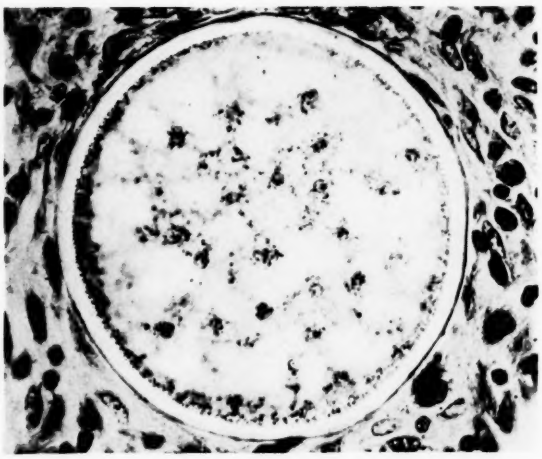

FIG. 8

FIG. 6. LOW POWER FIELD TO SHOW A GROUP OF PARASITES IN VARIOUS STAGES OF DEVELOPMENT

In three of the young forms can be seen the single sharply defined nucleus with its karyosome. There is one partially developed and one mature sporagium. Eosin-methylene blue stain. × 80.

FIG. 7. ABSCESS CONTAINING REMNANTS OF A RUPTURED SPORANGIUM

The convoluted structure is the disintegrating sporangial wall. A fragment lies free within the bend above. The lighter stained zone bordering the wall above and to the left is made up of undeveloped spores. Such spores form a crescentic marginal layer about the lower hemisphere of the matured sporangium, as can be seen in figures 1 and 2. Their location suggests that the wall has turned inside out. Giant-cells are present about the periphery of abscess cavity. Iron hematoxylin stain. × 80.

FIG. 8. TROPHIC FORM IN INTERMEDIATE STAGE OF DEVELOPMENT

Twenty-eight nuclei are present in the plane of section. Eosin-methylene blue stain. × 690.

recovered. Smears of the thick mucous secretion bathing the polyp and the floor of the fossa revealed the presence of large numbers of typical spores which are readily stainable with simple dyes (fig. 1). On account of the small amount of material available, no animal inoculation was attempted.

Due to the patient's recent absence from home it is not known whether there has been recurrence of the growth but the parents state that none was evident on his departure in December, 1930.

Microscopic findings

The growth is a papillomatous tumor with stroma denser than that found in the usual nasal polypus. There is suggestion of a central supporting framework free of parasites and of a looser surface zone of varying depth in which many parasites are present. There is a generalized low grade inflammatory infiltration together with frequent small scar areas. Concerning the parasite, my findings are in agreement with those worked out in much greater detail by Ashworth. Not all the stages described by him have been found, our particular deficiency consisting in the absence of examples showing the minutiae of the nuclear divisions. With this exception, the sections do however allow a satisfactory reconstruction of Ashworth's developmental cycle and as far as can be determined all the details of structure and measurement are found to be as he described. The organism is believed to be morphologically identical with that of Indian origin. The youngest trophic stages of the parasite are spherical or slightly oval bodies 6 to 9 micra in diameter that possess a relatively thick clear-cut refractile wall, a distinct nucleus and karyosome. They are found typically within the stroma tissue, particularly within the subepithelial zone. As the organism enlarges the cytoplasm becomes heavily granular and when a diameter of some 40 to 50 micra is reached, nuclear division begins. The daughter nuclei repeatedly subdivide while the entire cyst continues to increase in size. Cytoplasmic division finally occurs at a cyst diameter of around 120 to 140 micra, and there results a sporangium filled with great numbers of spores. These expand and distend the sporangium to a final diameter of 250 to 300 micra. During the middle term of development there appears an annular thickening of the wall about a focus that is destined to become a pore through which the spores will be discharged after maturation. The pole thus established is directed toward the surface of the polyp (fig. 2). The spores lying nearest this pole enlarge and differentiate while those at the opposite pole remain small and immature. The adult spores are round or oval, the former averaging 7 or 8 micra in diameter, the elongated forms reaching 9 or 10 micra or in some cases even 12 or 13 micra in length. They are outlined by a distinct membrane and contain a nucleus with prominent karyosome. The cytoplasm is distinct in the earliest recognizable phases, but soon becomes vacuolated and then obscured through an increasing development in it of round bodies said by Ashworth to consist of proteinaceous material and called by him "spherules." It is these

spores that are found in the nasal discharge as above noted and they may be seen in great numbers in sections lying upon the epithelial surface particularly in its crypt-like folds.

DISCUSSION

No other phases than those here sketched have been observed. As far as is now known they comprise the entire cycle of development within the human host. It is probable that the indicated cycle is continuously repeated through transformation of the spore into the encysted phase, but it is not known exactly how this takes place. Neither is it known how original infection occurs, nor how auto-inoculation at new sites is to be explained. From analogy with the known cycle in the sprozoa, the earlier students searched for an intracellular sporozoite development. They misinterpreted the spore structure and considered all its contained bodies as sporozoites or as a cluster of spores contained within a spore morula. Ashworth corrected these views and it was his great contribution to demonstrate that the so-called spore morula is a unit rather than a collection of equipotential spores. He believed that the spore develops directly into the trophic form, an opinion with which I agree. Just where or how this occurs is still a problem.

The question is bound up with that as to where the discharge of the spores normally occurs. According to descriptions which have been available, it appears to be assumed that the sporangia mature normally within the subepithelial tissues and discharge their contained spores directly into the stroma of the polyp. In our preparations this has seemed to be an accidental rather than a normal event. Most of the matured sporangia are found at the surface, where they are so situated that they can discharge their contained spores into the nasal cavity. Often they lie free within spaces outlined by infoldings of the surface epithelium and successive stages can be traced in the extrusion of the entire body through the covering epithelium from the site of maturation in the upper stroma. Ashworth describes such matured sporangia as occurring in the nasal discharge. Spontaneous discharge of spores could be seen in some cases, and unopened forms could

be made to rupture and discharge by being placed in water. The observation is of interest in connection with the suggestion from Indian observers that the infection may be water-borne.

After the spores are discharged upon the surface reinfection would require penetration of the epithelium. Such an event must be rare. The spore is a body distinctly larger than a red cell. There is no evidence that it is ameboid, indeed its structures suggest rather that it is a rigid body. Search of the present material supports the conclusion of others that intra-epithelial forms are extremely rare. We have found two spores lying within the lower zone above the basal line of nuclei. One early trophic form 8 by 9 micra in diameter is undoubtedly embedded at the middle level and a larger form 37 by 40 micra in diameter is probably intra-epithelial although open to some question of tangential sectioning. As compared with the great abundance of all stages of the parasite in the subepithelial tissue, these bodies are probably to be regarded as accidential imprisonments and it is not believed that continuous reinfection takes place by way of the transepithelial route. In the original infection and in the occasional instances in which auto-inoculation has occurred at new sites, it is probably necessary that the organism find its way in through an open wound of the epithelial covering.

It is possible that reinoculation and the initiation of new cycles may take place as the result of leakage of discharging spores into the upper stroma as surface sporangia discharge. When the ripening sporangium enlarges and bulges upward, the overlying epithelium is thinned and may disappear over an area large enough to allow of the eventration of the entire body. Again it may open only in a narrow sector over the site of the pore. As the pore opens and spores are discharged, some may make their way beneath the unseated epithelium in the space between it and the cyst wall, whence they are carried into the tissue by lymph currents. Suggestion of such an event may be seen in the sections, but may of course be artefact. Or again, the spores may find lodgement in the stroma within the fresh wound resulting from the expulsion of a discharging cyst (see fig. 3).

Finally, new generations may develop directly from spores

liberated into the deeper tissue by the rupture of cysts located beneath the surface. This has been the explanation of the writers beginning with Seeber and O'Kinealy and is perhaps the reason why the sub-surface rupture of the sporangium has been accepted, at least tacitly, as the normal method of discharge. In the present study this sub-surface rupture has appeared the most interesting phenomenon exhibited but, as already stated, it is interpreted as an accidental occurrence as contrasted with a normal surface rupture and discharge of the sporangium. When it occurs the spores, possibly accompanied by a little mucinous embedding material, escape directly into the stroma. A microscopic abscess results, starting about the open pore (fig. 4). Unless this abscess is close enough to the surface to open at once upon it, liquefaction extends entirely around the sporangium so that it lies free in a space crowded with neutrophiles and large mononucleated phagocytes. Spores, many of them in various stages of disintegration, lie in the space and the phagocytes are filled with basic or acid staining particles.

Occasionally a distintegrating spore itself can be recognized within a large phagocyte. Eventually the cyst wall spreads wide open and becomes looped or contorted as though it were an elastic structure that has been relieved of tension. Vaughan, in describing O'Kinealy's[5] specimen remarked this suggestion of "a certain degree of elasticity." Very often the convex surface of the distorted membrane is covered by a thick layer of immature spores, such as may be seen normally at the basal pole of the unruptured sporangium. There is here a suggestion that the cyst has turned inside out. In young cysts that have failed of development, or in those that have ruptured, the wall may fragment and particles may be found enclosed within giant-cells (fig. 3). Giant-cells appear also about the abscess margins to assist in the removal of debris. Resolution of the abscess is followed by contraction of the embedding tissue and the remaining central space is filled by the formation of a loose, often edematous, scar tissue containing a network of thick collagen fibrils, but relatively few active fibroblasts. Within it may be found occasional early trophic forms of the parasite. Such scar

areas in various stages of organization may be seen scattered everywhere through the stroma.

The cyst wall of the early trophic form is believed by Ashworth and others to be chitinoid. In later development there is laid down inside it a thick layer of material "indistinguishable from cellulose." The resulting stratification is often clearly defined in stained specimens. I have found that the chitinoid layer is stained intensely by elastic tissue stains so that it is sharply defined in the youngest forms down to diameters of 6 micra while in the adult stages it persists as a delicate outer layer and is still recognizable in the collapsed remnants lying within an abscess cavity. Orcein has been especially useful for this definition. The whole wall is stained intensely blue by Mallory's aniline blue connective tissue stain and this stain has also been useful in searching for the youngest trophic stages. The most generally useful stain has been eosin-methylene blue. It affords good differentiation of the finer details of the spore structure, the basic stained nucleus standing out in sharp contrast to the acid stained spherules (see fig. 5).

The old suspicion that reseeding of the tissue takes place from the spores liberated within the abscess cavity is believed to be well founded. Some of these spores probably resist the destructive activity of the hosts' cells and survive to be carried into the marginal tissue, or to develop within the scar tissue laid down at the abscess site. The adult spore, as it lies within the sporangium or upon the epithelial surfaces of the polyp, is outlined by a thin membrane or capsule that is sharply defined in eosin-methylene blue preparations and unstained, or at most merely indicated, by orcein or the connective tissue stain. In the trophic forms, from their earliest recognizable to their oldest stages, the latter two stains bring out with great sharpness the thick wall that is the main distinguishing feature of the encysted, as distinguished from the spore, stage. Within the abscess cavity, as occasionally within surface forms, there can be made out a thickening of the capsule and an increased density of its substance. As noted by Ashworth, this change is accompanied by a decrease in the size, and finally in the number of the spher-

ules. While no satisfactory series of forms has been traced, it appears probable that this "transformation" proceeds directly to the complete disappearance of the spherules and the acquisition by the capsular substance of orcein or aniline blue tingability with a median stage in which there is a well defined cytoplasm that becomes broken up into a dispersed granular state as the whole body becomes smaller and the trophic form is finally developed. These early trophic forms occur within recently developed scar areas and have probably developed here.

In view of the rarity of (recognized) cases of rhinosporidial infection, their wide geographical distribution is of extreme interest. There seems no reason to doubt that the organism reported from different parts of the world is a single form. Unrecognized cases have undoubtedly occurred but even with this possibility in mind it is still evident that successful inoculation of man must be characterized by a remarkable discontinuity. Our patient had always lived in a small village of a few hundred inhabitants where almost if not all disability comes under the observation of the physician who first recognized the present lesion. He recalls only one other nasal polyp among the population of the community. This was seen and removed ten years ago but the patient's subsequent history and present whereabouts are unknown. Except for this there has been no case suggesting a similar condition. None of the other members of the family show any evidence of infection nor does inquiry reveal any in relatives or playmates. The patient has never been in contact with the larger domestic animals except possibly during his six weeks visit to a farm eight years ago, when he was but five and one-half years of age.

SUMMARY

A case is reported of rhinosporidial infection in a nasal polyp. The patient was a twelve-year old negro boy born in Georgia and resident since the age of four in a small coal-mining village in Alabama. The source of the infection is unknown, no other similar condition having been observed in the community for at least ten years. The case illustrates the remarkable discon-

tinuity of the infection, of which more than forty known cases reported during the past thirty years have been observed in three widely separated localities, India, Argentina and the United States.

Preoperative diagnosis should be made without particular difficulty through recognition of the characteristic spores in the nasal secretions.

REFERENCES

(1) Ashworth, J. H.: On Rhinosporidium seeberi, with special reference to its sporulation and affinities. Tr. Roy. Soc. Edinburgh., **53**: 301–342. 1923.

(2) Doflein, F.: Lehrbuch der protozoenkunde. 3rd. Ed. 1043 pp. Jena, G. Fischer. 1911.

(3) Lincoln, M. C., and Gardner, S. M.: A case of Rhinosporidium seeberi in a resident of the United States. Arch. Path., **8**: 38–45. 1929.

(4) Minchin, E. A., and Fantham, H. B.: Rhinosporidium kinealyi, n.g., n.sp., a new sporozoon from the mucous membrane of the septum nasi of man. Quart. Jour. Micro. Sci., **49**: 521–532. 1906.

(5) O'Kinealy, F.: A microscopic section of localized psorospermosis of the mucous membrane of the septum nasi. Jour. Laryng. London., **18**: 375–378. 1903. Also see **19**: 93–94. 1904.

(6) Seeber, G. R.: Quoted by Ashworth.

(7) Weller, C. V., and Riker, A. D.: Rhinosporidium seeberi; patholgical histology and report of third case from United States. Amer. Jour. Path., **6**: 721–732. 1930.

(8) Wright, J.: A nasal sporozoon (Rhinosporidium kinealyi). N. Y. Med. Jour., **85**: 1149–1153. 1907.

GLYCOSURIA AND THE GLYCEMIC TOLERANCE CURVE

A Review

EDGAR T. HERRMANN

School of Medicine, University of Minnesota and Miller Hospital Clinic, St. Paul, Minnesota

The chief object of this review is to point out the practical application of the sugar tolerance curve as an aid in the differential diagnosis of glycosurias of uncertain nature arising in man. To this end a survey of the factors determining and modifying such a curve will be discussed as they have been obtained by various investigators throughout the world within the last twenty-five years.

Any discussion of glycemia obviously necessitates inquiry into two factors that regulate the concentration of blood sugar, the threshold on the one hand and the capacity of the tissues to remove sugar from the blood on the other. Claude Bernard, during the nineteenth century, brought the problem into clear relief. In 1913, Aage Jacobsen,[35,34] working in Faber's clinic, conducted a series of investigations on the sugar threshold. Others followed him, notably Faber, Karen Marie Hansen,[15, 14, 16, 13] Hagedorn,[25] Hatlehol,[30] MacLean,[40] and Traugott.[51] The definition of threshold is that concentration of sugar in the blood beyond which it appears in the urine. Jacobsen fixed this at 160 to 180 mgm. per 100 cc. of blood for normal persons, using Bang's micro-method of determination. Higher or lower values are not infrequently found, Hatlehol finding figures as low as 116 and 126 mgm. per 100 cc. in normal persons. Faber and his school defined the properties of the threshold in the following manner: it is constant in every individual, independent of age and diet, the presence of diabetes or its duration. Its position differs, however, in different individuals. Faber thus conceived the threshold to

be an "inherent quality in the individual, a constitutional quality." It may change in the individual under certain conditions such as phloridzin administrations or pregnancy. Most investigators concur with this definition in so far as it applies to normal threshold. Traugott,[51] however, said that a renal threshold for dextrose cannot be determined, nor does he feel that the kidney has a certain point of permeability for dextrose. He contended that the so-called renal factor in glycosuria was only an apparent one, being the expression of processes taking place outside the kidney. Following the work of Lepine he felt that alimentary hyperglycemia in normal people is not caused by a passage of sugar through the gastro-intestinal tract by way of the liver into the blood, but is chiefly caused by the sugar elaborated within the liver in response to the alimentary stimulus. This sugar is not passed by the kidney since it probably circulates in the blood in a bound form. Very exceptionally sugar does pass from the intestine into the blood stream causing a glycosuria, but in that case the sugar value for plasma is significantly higher than that of whole blood whereas normally he found plasma and whole blood to contain the same sugar values. Thus he felt that he had explained, on extrarenal grounds, why certain cases of alimentary hyperglycemia show glycosuria and others do not as well as why the glycosuria, when it occurs, is independent of the height of the blood sugar.

This called to mind Benedict's glycuresis and suggested the interesting theory on the nature of the glucose-threshold propounded by Folin and Berglund,[18] an excerpt from which is hereby quoted:

> There can be no doubt as to the existence of some mechanism by which the excretion of glucose is normally absolutely prevented. If we here venture to advance something approaching a new explanation we realize that it may be wholly wrong, but we believe that it should be at least partly correct. Each of us independently has in former researches seen and emphasized the avidity with which tissues may absorb materials from the blood. And throughout this investigation we have agreed that it is absorption by the tissues rather than the glycogen formation which prevents excessive accumulations of the sugars in the blood. With reference to glucose we assume that the tissues always contain at least as high a concentration of free sugar as the blood plasma and probably more.

The glycogen formation need not begin until the tissues have begun to possess a much higher concentration than that present in fasting. Much absorbed sugar can thus be distributed without any large increases of the sugar in the blood. But the kidneys receive their quota of sugar, just as do the other tissues, and this increase of sugar does not involve the slightest degree of strain. The strain comes only when the holding capacity for free sugar is reached and when the glycogen formation must come into play to keep the sugar concentration within normal limits. The speed of glycogen formation is of a much lower order than is the earlier process of merely absorbing the sugar from the blood. At this stage, therefore, the sugar backs up in the blood and *the holding capacity of some tissues including the kidneys is exceeded.* As a result of the strain thus produced the kidneys are finally compelled to make use of a more efficient process than the glycogen formation for reducing the sugar concentration in the kidney cells and the elimination of sugar suddenly begins. That a real local strain has preceded the escape of the sugar is indicated by the fact that the sugar excretion once begun does not stop as soon as the blood sugar has fallen below the threshold, but, in fact, continues until the level of the blood sugar has gone away down, even to subfasting values (hypoglycemia). It is as if there had sprung a leak for sugar, a leak which cannot be immediately repaired. Yet the total amount of sugar eliminated need not be very large except in clinical or experimental diabetes, because other tissues continue to absorb sugar as well as to make glycogen.

We cannot here develop this interpretation in more detail because of its many ramifications. The essential point is that it makes clear why increasing concentrations of sugar in the blood below the threshold values involve no strain and no elimination of glucose. From our interpretation it also follows that excretion of glucose, as in emotional glycosuria and renal glycosuria, does not represent a finely adjusted normal process analogous to the excretion of sodium chloride or of waste products. It is also in harmony with the belief in the importance of not permitting diabetic patients to excrete any sugar at all, if it possibly can be prevented.

Hamman and Hirschman,[28] felt that there was displacement of the threshold in diabetes and nephritis, the latter usually having a high threshold. Faber felt that this was due to the method of threshold determinations and on the basis of the method developed by himself, A. Norgaard and Karen Marie Hansen, seemed justified in his conclusions. By determining cutaneous (capillary or precapillary) blood sugar values every five minutes after the ingestion of two dissimilar doses of glucose, amounts of 35 and 25 grams for example, the threshold value appeared between the two.

One of Faber's charts is herewith reproduced (fig. 1*) in which it is seen that whereas 25 grams of glucose caused no glycosuria, 35 grams did, though sugar appeared in the urine only when the glycemic curve had begun to fall from its peak. Obviously then, the threshold here lies between the peak of the two curves.

Normally, the threshold usually varies between 0.160 and 0.180 per cent though it may, even in diabetes, be as low as 0.116 per cent or as high as 0.240 per cent. Karen Marie Hansen held that in normal individuals there is no break over the threshold even if 200 to 400 grams of sugar are ingested but Malmros, showed that in persons

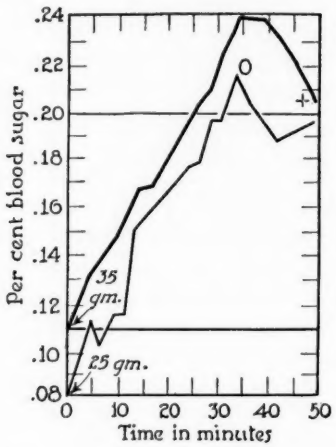

FIG. 1. REDRAWN FROM FABER

given 3 grams of glucose per kilogram there was a retention of 40 per cent of the ingested glucose in the stomach at the end of two hours. Thus the dictum that there is no assimilation limit for glucose in normal persons may not be altogether pertinent. The fact that sugar does not appear in the urine after its ingestion in large amounts, means that the body tissues present a mechanism of withdrawl that is extremely effective under ordinary circumstances. Karen Marie Hansen termed this exchange between tissues and capillaries the acceleration capacity of the organism,

* Except where otherwise stated the figures are constructed from data of investigators mentioned and are not reproductions.

a mechanism whose function is notably disturbed in true diabetes mellitus.

Since the position of the threshold determines the type of glycosuria and the course of the glycemic curve depends on the functional integrity of the removal mechanism, the best means of determining these factors becomes diagnostically important. On this account the choice of method is of fundamental importance, and choice of arterial (capillary) or venous blood sugar determinations arises. In this country most sugar determinations have been made on venous blood, whereas abroad and particularly

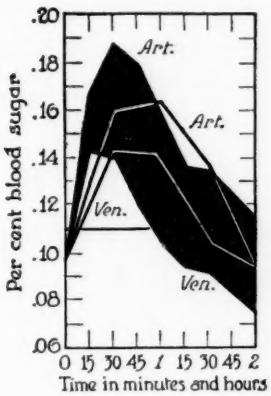

FIG. 2

in the Scandinavian countries, the use of arterial blood is more common, due perhaps to Bang's[3] introduction, of a practical micro-method for its determination. The arterial method should be the method of choice for the following reasons: first, the blood sugar percentage in the renal arteries must be the decisive factor in the occurrence of glycosuria; second, arteriovenous differences up to 50 mgm. are not unusual from thirty to sixty minutes after glucose ingestion (Friedenson et al.[23]), third, venous values are lower than arterial values in alimentary glycosuria, and finally, catching the peak of the rise often makes removal of blood at five minute intervals imperative.

Normals for arterial and venous blood have been established on

a series of individuals both by Foster,[21] and in Benedict's laboratory.[5] Figure 2 shows the two curves superimposed, Foster's values appearing in solid black.

All investigators feel that in normal fasting individuals, arterial and venous blood specimens give practically the same values. In sixteen normal cases Cori et al[9] found the difference to average 5.5 per cent; Dorle et al,[10, 11] found practically no difference in the two, either after fasting or after food intake in normals or in people with hypertension, although in lues they found the venous sugar after fasting higher than the arterial sugar. They also found that after fasting, mild exercise caused an increase of arterial over

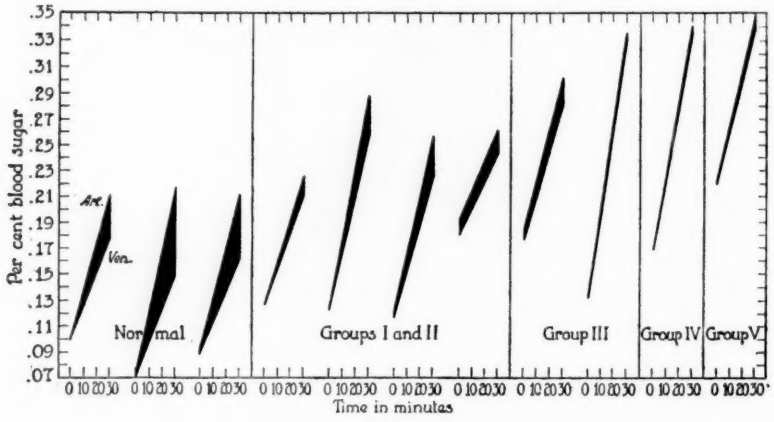

FIG. 3

venous blood sugar values. Practically all observers feel that the absorptive tissue mechanism accounts for the normal arteriovenous difference.

Investigations of the last few years point to the conclusion that, at least in diabetes, the presence or absence of arteriovenous difference is important. Figure 3 is constructed from figures of Rabinowitch,[44] who felt that the rate of utilization of glucose parallels the severity of the disease, which means that arteriovenous difference is greater, the greater the rate of utilization. By utilization he meant the combined mechanism of oxidation and storage. In figure 3 there is given first a group of normals. Groups I and

II represent transient or postprandial glycosuria, probably non-diabetic; group III represents people with persistent glycosuria and fasting hyperglycemia. In group IV are patients of diabetes who show acetone bodies in the urine but who get on well without using insulin. Group V represents an exaggeration of group IV and needs insulin to meet daily requirements. Roughly, two facts stand sharply defined as one observes the graphs, namely, that the fasting blood sugar tends to rise throughout and that the arteriovenous difference tends to disappear.

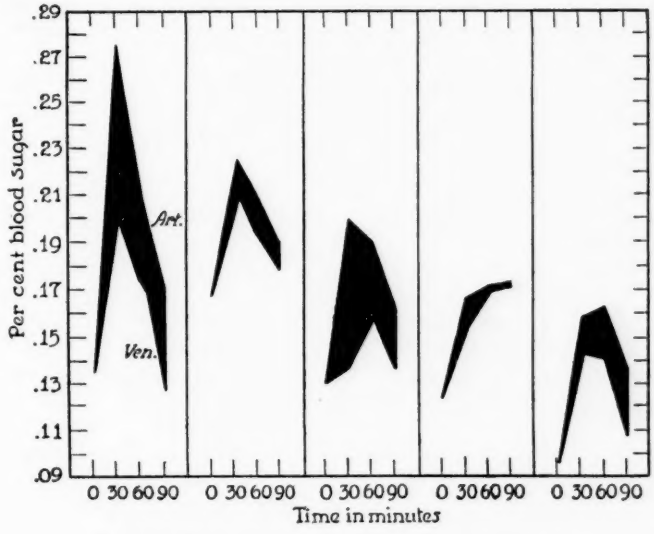

FIG. 4. REDRAWN FROM FRIEDENSON ET AL.

The importance of the arterial curve is emphasized in figure 4, reproduced from Friedenson, Rosenbaum and Thalheimer,[22] in which the lack of rise of the venous curve strongly emphasizes its unreliability as a method of tolerance investigation. At the height of the curve one observes differences of 50 to 70 mgm. which serve to invalidate the venous method in the doubtful case.

The rest of this discussion is based on the sugar content of arterial blood, sugar here meaning reducing substance in the blood. Folin and Svedberg[19] concluded that the blood contains ferment-

TABLE 1

NORMAL BLOOD SUGAR VALUES

AUTHOR	VALUES	MEAN	CASES	METHOD	REMARKS
	per cent	*per cent*			
Naunyn	0.07 –0.10	0.080	4	Abeles	
Klemperer	0.08 –0.11				
Liefmann and Stern	0.065–0.105	0.086	20	Knapp	
Weiland	0.077–0.101	0.090	6	Knapp	
Hollinger	0.065–0.100	0.090	10	Knapp	
Hollinger	0.068–0.089	0.080	5	Knapp	
Tachau	0.064–0.088	0.083	16	Knapp-Tachau	Age: 16–54
Tachau	0.061–0.084	0.078		Knapp-Tachau	Done on weight units
Frank and Bretschneider	0.074–0.110	0.084	4	Bertrand	In plasma
Frank	0.08 –0.11	0.095	15	Bertrand	In plasma
Frank	0.098–0.102		3	Bertrand	In plasma. Age, 76
Rolly and Oppermann	0.062–0.088	0.076	14	Bertrand	Fasting normal
Rolly and Oppermann	0.078–0.107	0.096		Bertrand	In plasma
Grigault, Brodin and Buzand	0.088–0.105	0.096	13	Bertrand	
S. P. Swart	0.06 –0.11			Bertrand	
Kowarski	0.05 –0.11			Kowarski	
S. Strouse	0.04 –0.12	0.084	61	Kowarski-Strouse	
Schirokauer	0.072–0.120	0.103	25	Bertrand-Mockel-Frank	In serum
I. Bang	0.10 –0.11		3	Bang (micro)	
Leire	0.06 –0.11	0.090	17	Bang (micro)	
Schumm and Hegler	0.073–0.116	0.092	22	Bang	
Thannhauser and Pfitzner	0.07 –0.09	0.08	3	Bang	Normal
Bing and Windelor	0.60 –0.113	0.096	16	Bang (micro)	
Ryser	0.063–0.105	0.086	21	Bang-Asher	Normal, fasting
Bing and Jacobsen	0.06 –0.12	0.099	16	Bang (micro)	Normal, fasting
A. Jacobsen	0.083–0.128	0.101	14	Bang (micro)	Fasting
M. Elzas	0.080–0.115	0.100	25	Bang	Normal, fasting
H. Staub	0.073–0.113	0.096	55	Bang (micro)	Normal, fasting
Hollinger	0.076–0.100	90.01	5	Lehmann, Modifiz.	
Forschbach and Severin	0.06 –0.09			Forschbach and Severin	

TABLE 1—*Concluded*

AUTHOR	VALUES	MEAN	CASES	METHOD	REMARKS
	per cent	*per cent*			
W. Benningson.....	0.063–0.100	0.0875		Forschbach and Severin	Volume, per cent
W. Benningson.....	0.06 –0.096	0.0833		Forschbach and Severin	Weight, per cent
Levis and Benedict.	0.09 –0.11			Picrat-Colorimetrie	
A. O. Gettler and W. Bauer.....	0.050–0.130	0.088	29	Picrat-Colorimetrie	3 hours after breakfast
Myers and Bailay..	0.09 –0.11			Picrat-Colorimetrie	
Salomon.....	0.09 –0.12		12	Picrat-Colorimetrie	Normal age, 17–18
Salomon.....	0.07 –0.12		20	Picrat-Colorimetrie	Normal and cured age, 20–48
Salomon.....	0.08 –0.15		10	Picrat-Colorimetrie	Weakness of age. No other signs
Wacker.....	0.12– 0.18			Wacker	
Watermann.....		0.100	6	Wacker-Watermann	
Reicher and Stein..	0.09 –0.15			Reicher and Stein	
Taylor and Hulton..	0.05 –0.150				

able sugar other than glucose which is not maltose or any other di- or polysaccharide.

Of the arterial methods in use the chief ones are those of Bang, Hagedorn-Jensen,[26] Folin,[17] or Benedict,[5] together with numerous modifications of them all. With every one, it is important to hold rigidly by the chosen technique, being careful to eliminate all sources of error, and checking from time to time against solutions of a known sugar content. At the same time, as shown by Pickard and Pierce,[42] observed values should be checked against the biometric curves for error worked out by them. Absolute accuracy is imperative since it has been shown that with all of the methods the fasting value varies between 0.07 and .11 per cent and that observed values of more than 0.11 per cent are sug-

gestive of diabetes. Thus Liefmann and Stern,[39] Bang,[2] Staub,[48] Hagedorn,[25] Hansen,[29] Gray,[24] reported these figures as normal variants. Very occasionally values up to 0.115 to 0.12 per cent are found. Gray said in effect that the fasting value in 431 apparently healthy persons averaged 0.09 per cent. The unusual figures, however, of 0.12 per cent to 0.16 per cent were reached in as many as 7 per cent of these normals, thus leading to the alternatives: clinical judgment of normal metabolism is untrustworthy, or a considerable number of normals exhibit suspiciously large fasting figures. Punschel,[43] found fasting values in young people that averaged 0.094 per cent, using Bang's method. In individuals between fifty-eight and seventy years of age he found an average of 0.106 per cent, while the values in those between seventy and ninety-one years of age averaged 0.110 per cent, one of the latter reaching 0.133 per cent. Malmros,[41] however, never found fasting values above 0.11 per cent in people between fifty and seventy years of age. The practical unanimity of the fasting range is well illustrated by the tabulation collected by Punschel. Increased fasting sugar values, exclusive of diabetes, are found also in febrile diseases, especially malaria, in the latter weeks of typhoid and in severe cases of scarlet fever (Andresen and Schmidt.[1] Liefmann and Stern found hyperglycemia up to 0.28 per cent in lobar pneumonia without glycosuria. Williams and Humphreys[54] found a mild elevation of blood sugar from 0.12 per cent to 0.16 per cent in nine cases of carcinoma, a marked increase in the last stages of nephritis and a moderate increase in cases of hypertension.

The technique of the tolerance test as employed on most of the curves here recorded has been worked out chiefly by the Scandinavian investigators and the modification presented by Malmros seems the most satisfactory. He gave the patient, twelve hours after the last meal, the equivalent of 1 gram per kilogram (of body weight) of glucose in a 10 per cent solution on an empty stomach. Arterial sugar determinations were then made at five or 10 minute intervals over a period of two or three hours. Hagedorn[25] earlier utilized this method and by means of it worked out what he termed the assimilation figure, which is the relation between the

amount of glucose administered and the amount that has circulated through the depots of the organism during the experiment in excess of the amount circulating before glucose was given. In normal individuals this figure is almost always above thirty. Malmros[41] found that he got the same curve with 0.5 gram per kilogram as with 1 gram, but not so high a rise when using 0.33 of a gram per kilogram. It is important to note that when he used 3 grams per kilogram of body weight he found 40 per cent of the ingested sugar present in the stomach at the end of two hours, yet the blood sugar curve fell to normal in two hours, which he interpreted as meaning that the withdrawal of sugar from the blood had been more rapid than the entry of sugar to the blood from the intestinal tract. Thus the course of the curve, in his opinion, depended chiefly on the withdrawal mechanism into which might enter the factor of absorption per unit of time. Therefore the higher curve obtained in diabetes on larger amounts of ingested glucose must depend on the withdrawal mechanism. The following quotation from Malmros[4] gives the exact technique of the procedure:

> The experimental subjects had been on ordinary mixed diet for several weeks preceding the experiment. The determinations were made in the morning 12 hours after the previous meal. The person to be examined had been told to empty his bladder immediately on awakening in the morning. The bladder was emptied once more immediately before taking the glucose and once every half hour the next 2(–4) hours. Blood was taken fasting (immediately before the glucose) and afterwards once every 5–10 mins. during the first hour. The samples were generally taken most often (every 5 mins.) from 20–40 mins. after the glucose ingestion. During the 2nd hour the samples were taken as a rule every 15 mins. sometimes every 10 minutes. In a number of cases the examination lasted 3–4 hrs. In those cases samples were taken every half hour after the second hour. Duplicate samples were always taken, often 3 samples fasting. . . . . The dose of glucose ingested was 1 gm. per kgm. body weight; it was given as a 10% water solution. Merck's preparation, grape sugar, extra pure, anhydrous was used.
>
> To avoid nausea the glucose solution has been cooled and flavoured with the juice of half a lemon. Each person took the sugar solution easily (in the course of 1–5 mins.), and none was nauseated. The time is counted from the moment when the experimental subject began to take the glucose solution. During the experiment the subject was sitting in a chair or lying in bed.

The frequent intervals, particularly during the first hour, are important because usually the height of the rise occurs between the first twenty and forty minutes.

Before continuing with a discussion of this type of curve, a brief reference to other types of diagnostic curves may not be out of place. Of the type obtained when the patient is given glucose

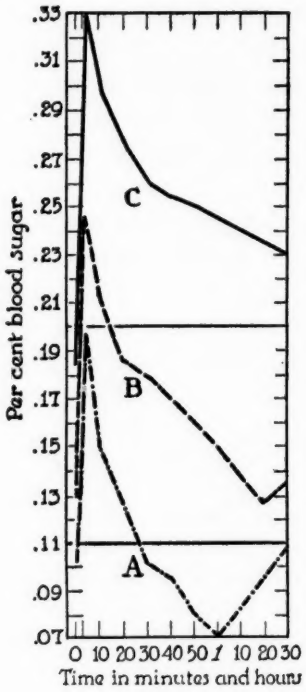

FIG. 5

by vein, one of the most satisfactory is perhaps that used and reported by Wislicki.[55] He gave 20 cc. of a 40 per cent dextrose solution by vein and the type of curve is reproduced from his data in figure 5. A, represents the normal, in which the fasting value is reached within thirty to forty-five minutes, B is the reaction of the mild diabetic and C that of the severe diabetic. The intravenous use of dextrose avoids alimentary sources of error and

possesses as well the advantage of rapidity and usability in cases in which the alimentary route cannot be employed.

The type of curve used by Seyderhelm[46] in von Noorden's clinic is termed the "Tages profil" of the blood sugar. This supplies a picture of the type of curve existing throughout the day and the technique is as follows: blood sugar test is taken before breakfast, again before lunch and finally before dinner, the patient fasting during the test. Normal individuals and diabetics receive as car-

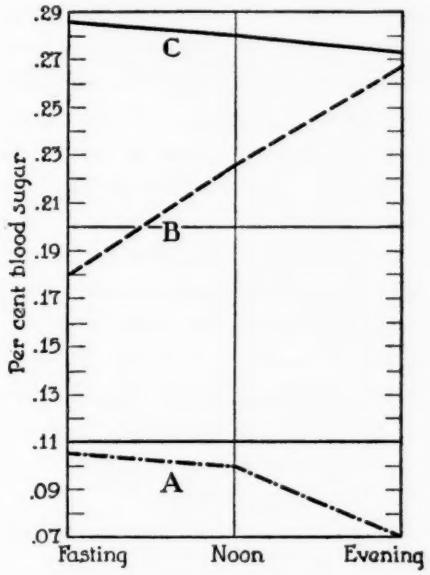

FIG. 6

bohydrate thirty grams of white bread on three occasions, first for supper on the night before, second for breakfast on the morning of the test and third for lunch on the day of observation. Repeated controls showed that in individual cases the carbohydrate portions could be varied, thus the morning dose could be given as two breakfasts of 15 grams each, but the second breakfast must be given two or three hours before the next blood sugar test. It was found, as shown in figure 6, that in normal individuals (curve A of the figure) the combined curve of the three blood sugar readings

depended upon the height of the blood sugar after fasting. Investigations showed that when the sugar value lies under 0.100 per cent the noon value often shows a slight rise. A marked fall of blood sugar value was never observed in the noon value of this group, while in the normal individual the evening value rarely rose above the fasting value. Curve C shows a graph from cases of diabetics in which insulin had not been given. In these the noon blood sugar was always lower than the blood sugar after fasting and continued study showed these to be cases in which insulin treatment was not necessary. Curve B is a graph from a group of cases in which the noon blood sugar showed a rise and in which insulin treatment very soon became a necessity. Many cases in this group also showed acidosis. From this procedure Seyderhelm concluded that a case with a noon fall of sugar has a good prognostic outlook. He further pointed out that the absolute height of the fasting value plays no rôle in the prognosis since this value depends on previous carbohydrate intake. Noon and evening values, however, seemed to him to indicate the ability of the pancreatic islands to form sufficient or insufficient amounts of insulin. If noon values sank particularly low the prognosis was always particularly favorable, these cases needing no insulin. A still greater evening fall in these cases was likewise a favorable sign. This type of "daily profil" of the blood sugar curve possibly has clinical application.

At this point one may also mention the diagnostic procedure employed by John.[36] He felt that if a blood sugar was taken twelve hours after the last meal, and if then a patient was given a heavy carbohydrate meal, it was only necessary to get a blood sugar determination exactly three hours afterwards, to settle whether or not the person was diabetic. If the blood sugar was above normal at this time the case was one of diabetes irrespective of whether or not sugar appeared in the urine.

Figure 7 shows the normal values obtained by Malmros[41] and Rabinowitch,[45] the former using the arterial method, and the latter using the venous method of blood sugar determination. The solid line represents Malmros' mean arterial value, the dotted one that of Rabinowitch. The greatest arterial variation (usually

within fifteen to thirty minutes) is up to 0.20 per cent and down to 0.11 per cent in two hours. Although these are Malmros' figures obtained on forty normal individuals most other investigators agree with them. Rabinowitch felt that the highest venous rise should not be more than 0.18 per cent, as indicated by the horizontal dotted line. The position of the arrow in the figure indicates the point, in Malmros' cases, at which the stomach is empty of glucose. In practically all of the figures the normal arterial limits, 0.20 per cent and 0.11 per cent (the latter the upper fasting limit), are shown by fine and heavy, unbroken lines respectively.

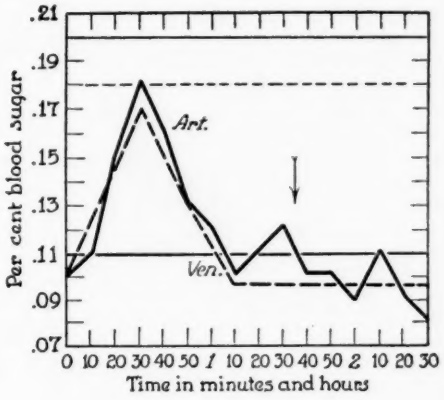

FIG. 7

Having determined the normal curve, the next question that arises is that of a classification of types of curves obtained in various types of glycosuria. By far the greatest amount of this work has been done by Scandinavians, particularly Jacobsen,[34] Faber,[15, 14, 16] Hagedorn,[25] Holst.[33] All these classified types of glycosuria on the basis of threshold and fasting Blood sugar values. Among various classifications that of Holst is chosen for reproduction. He recognized five types of glycosuria, based entirely on blood sugar examinations. There are two broad divisions, into the first of which fall cases of diabetes, defined by him as all those that show a fasting hyperglycemia, and into the second of which fall all cases of non-diabetic glycosuria. All of

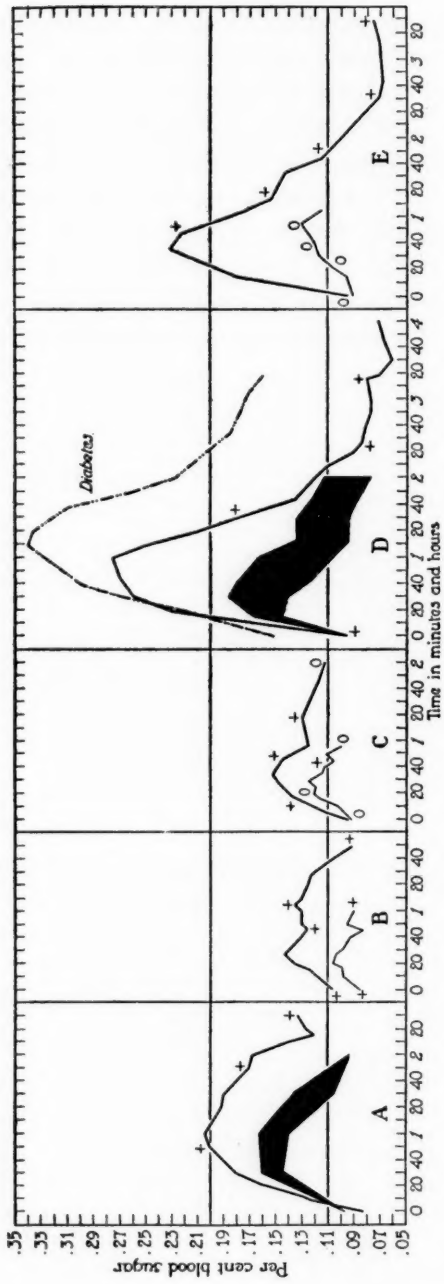

FIG. 8. MODIFIED AND REDRAWN FROM HOLST

the latter have normal fasting blood sugars without previous dietetic treatment. Figure 8 illustrates his five types of glycosuria and is a reproduction of Holst's figures with modifications showing two normal curves and one diabetic curve for purposes of comparison. Section A represents glycosuria with cyclic hyperglycemia. There is a normal fasting and threshold value, but the rise of the curve after glucose is greater than normal and there is glycosuria after meals. The black graph is included for comparison and shows Friedenson's[22] normal venous and arterial values. The plus sign means sugar in the urine at that point. Section B represents renal diabetes. The blood sugar after fasting is normal, the rise of the curve after glucose is normal but the threshold is lower than the blood sugar after fasting, resulting in a permanent glycosuria. The fine line represents the values obtained after the ingestion of 5 grams of glucose, the heavy line that obtained after 63 grams of ingested glucose. Section C represents cyclic renal glycosuria which differs from renal glycosuria only because the threshold is higher than the fasting sugar after fasting but lower than normal. The upper limit for a low threshold in these cases is taken to be 0.14 per cent. In this graph the fine line represents the curve obtained after 10 grams of ingested glucose and the heavy line that obtained after 50 grams. The morning urine is free of sugar but glycosuria occurs after carbohydrate meals. Section D represents transition cases with permanent glycosuria. The blood sugar after fasting is normal; the threshold is lower than the sugar value after fasting, and after glucose an abnormally high rise occurs. In this section Foster's normal venous and arterial values are represented in the black graph whereas the likewise interpolated dotted line illustrates a curve from a diabetic patient. Finally section E represents cyclic transition cases. This differs from D only in that the threshold is higher than the blood sugar after fasting but below 0.14 per cent. Thus the morning urine is sugar free but glycosuria occurs on feeding carbohydrates. In renal glycosuria as in transition cases the position of the threshold in certain cases can be such that the fasting blood sugar after fasting may be above or below it. Thus from time to time a

threshold value of 0.09 per cent to 0.10 per cent will give positive or negative test for sugar in a morning urine specimen.

The present stage of knowledge concerning threshold is still comparatively a matter of dispute, thus the above classification has points of inadequacy. Since its formulation, indeed, other schemes of division have been put forward, but on the whole it still presents a definitely usable set of data. Recently Malmros[41] suggested another which with slight modifications is as follows:

1. *Glycosuria innocens with low threshold value and continuous glycosuria.* Fasting blood sugar normal, urine containing sugar the day round even on empty stomach. 18 cases. Tolerance test curve normal except in 4 cases where slight hyperglycemia remains after 2 hours.

2. *Glycosuria innocens, cyclic glycosuria.* Blood sugar curve of indefinite type. Fasting sugar normal. Tolerance curve generally normal. In two cases hyperglycemia being of longer duration that normal. 1 case up to .21%.

3. *Glycosuria innocens with pathological alimentary hyperglycemia.* Glycosuria of cyclic type, powerful hyperglycemia in two cases of longer duration than normal. Fasting blood sugar normal.

4. *Cases with history of glycosuria negative on exam.* Fasting sugar normal. Tolerance curve normal in 1 case rising to .21%.

5. *Positive reduction test during lactation.* Use phenylhydrazin test and fasting sugar to avoid diagnosing diabetes when it is lactosuria.

6. *Cases with subjective diabetic symptoms, but normal fasting blood sugar.* The material comprises 6 men and 2 women.

The fasting blood sugar was normal in all the cases. Larger amounts of sugar were in no cases excreted with the urine. The urine was sugar free in some cases, even after glucose ingestion. On the glucose tolerance test a normal blood sugar curve was found in most of the cases, in case no. 53 the hyperglycemia was rather stronger than normal and in cases nos. 47, 49 and 52 of longer duration than normal.

7. *Glycosuria of uncertain nature; glycosuria innocens or diabetes mellitus?*

Such classifications postulate blood sugar curves obtained under standard controlled experimental conditions. It cannot be too strongly emphasized that various factors modify the course of the blood sugar curve, so much so that unless these are ruled out, the curve may present entirely abnormal characteristics, simulating pathological conditions. An accurate knowledge of these modifying factors is therefore an absolute necessity if the blood sugar tolerance curve is to be used as a diagnostic aid.

THE EFFECT OF THE ABSORPTION RATE FROM THE INTESTINE

This has already been discussed and Malmros has shown rather conclusively that a dose of 1 gram of glucose per kilogram of body weight insures accuracy on this score.

EFFECT OF PRECEDING DIET

This, probably the most important of all the modifying factors, has received comparatively little attention up to the present. Bang[3] was perhaps the first to show that rabbits starved for sev-

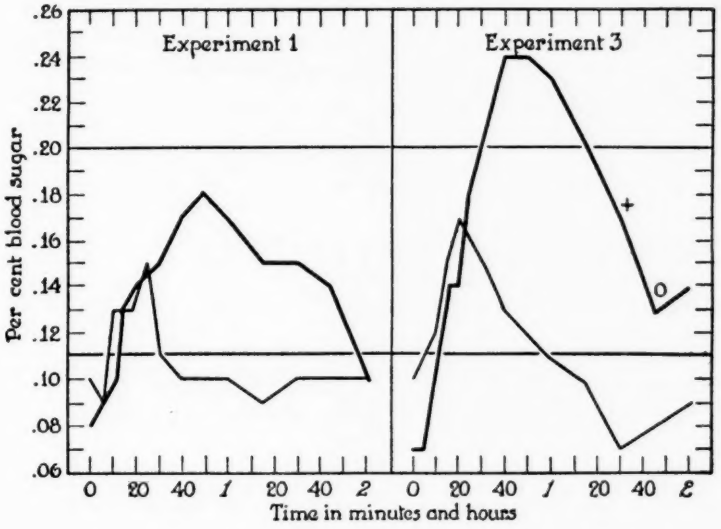

FIG. 9

eral days showed higher curves after glucose than fed rabbits. Traugott,[25] observed differences during the modified war diets, others likewise reporting altered curves with reference to diet. The following figures (fig. 9) show graphs constructed from some of Malmros'[41] data. In experiment 1 the fine line represents the tolerance curve obtained in the patient on a normal diet, while the heavy line represents the curve obtained after the same patient had been kept on a Petren diet for one day, the Petren diet being high in fat, low in carbohydrate and protein. In exper-

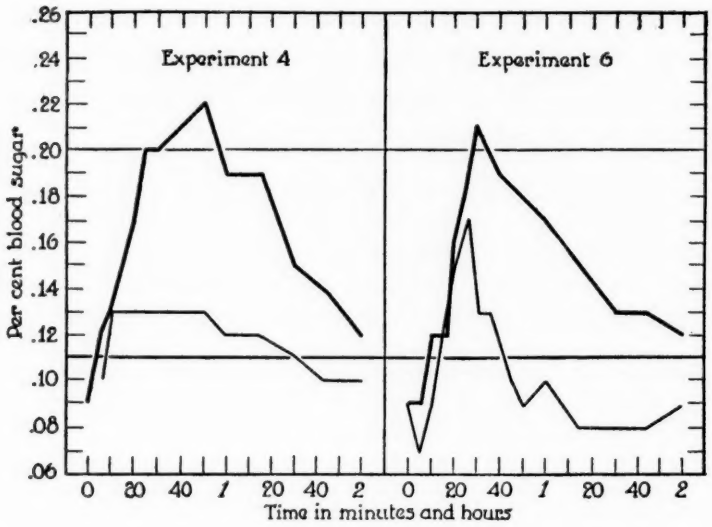

FIG. 10

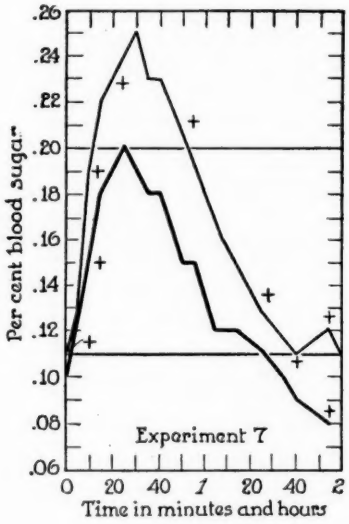

FIG. 11

iment 3 of the same figure the fine line again represents the normal curve, while the heavy line is the result of four days on a Petren

diet. Figure 10 represents the results of experiments 4 and 6, the heavy line in the former being obtained after a Petren diet of twenty-three days, the heavy line in the latter showing the modifications observed over a period of fifteen days. In figure 11 the fine line shows the curve found in a patient who had been on a restricted carbohydrate diet for thirty-one years while the heavy line represents the curve that was found in the same patient after

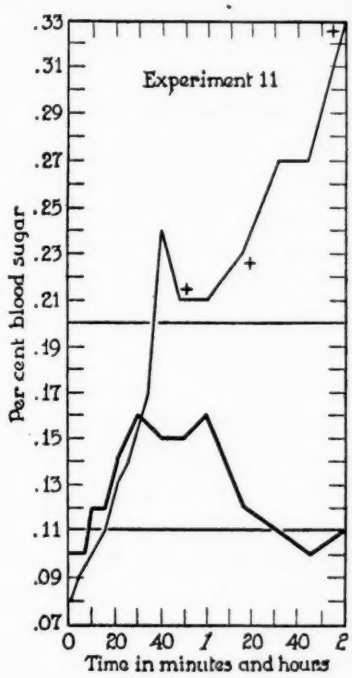

FIG. 12

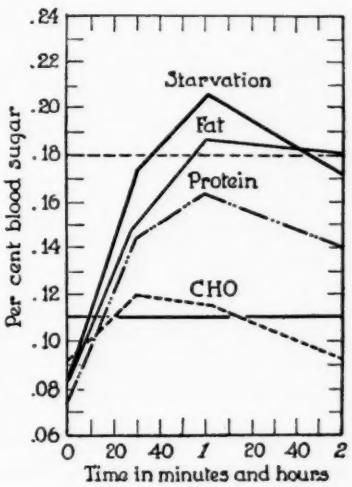

FIG. 13

two weeks on an ordinary restricted diet. Figure 12 shows the results of experiment 11. The fine line portrays the glucose tolerance curve after six days of fasting and the heavy line the curve in the same person obtained after ten days on an ordinary unrestricted diet.

In this country Sweeney[49] showed that the three major foods all affect the tolerance curve differently. Figure 13 portrays Sweeney's results as modified by fats, carbohydrates, proteins and star-

vation, when the diet for two days previous to the test has been restricted to the foods named. These are venous and not arterial curves. Of the foods, fat shows the highest rise and carbohydrate the lowest, while starvation produces the effect observed in Malmros' excellent example. Together with Staub, Sweeney postulated the activation of an intermediate hormone which in turn stimulates the production of insulin. Thus when dextrose is taken a hormone is produced which stimulates the production of insulin and the result is only a moderate rise in blood sugar, whereas in diabetes, although the hormonic response is normal after the ingestion of dextrose, the amount of insulin that is produced is insufficient to store the dextrose as glycogen resulting in hyperglycemia and prolongation of the curve. In the patients receiving fats the activation of the insulin stimulating hormone has been reduced, thus again resulting in hyperglycemia. The same thing is true, from his point of view, in starvation; such theoretical tenets are at present, of course, far beyond any observed data.

Various ideas have been advanced to explain changes due to diet. Thus Elias,[12] using rabbits, turtles and dogs, showed that relatively small amounts of acid have a mobilizing effect on the glycogen of the liver, and that adrenalin or the adrenal glands have no part in the resulting hyperglycemia or glycosuria. The action of the acid is directly on the liver cells, glycogen for the most part leaving the liver cells as intact glycogen under its influence. Haldane[27] showed that in acidosis produced by ammonium chloride coincident with normal administration of carbohydrates there is failure to store glucose but not to oxidize it. Barrenscheen[4] in a series of experiments conducted on rabbits and dogs demonstrated that under controlled experimental conditions dextrose and levulose formed glycogen directly in the liver, while this was not a direct process with maltose and galactose. He further showed that after extirpation of the pancreas perfusion of dog livers with the blood of normal animals failed to cause direct synthesis of dextrose into glycogen which is of interest in view of Cori's work. Staub asserted in the first place that the glucose fixation ability of the normal liver is

a very variable one, in which point of view he was upheld by Forsgren[20] and his associates, who said that the liver has rhythmically acting functions that are to a certain extent independent of the supply of nutrition, that is, that it possesses an assimilating and dissimilating stage with reference to glucose.

Staub further felt that the ingestion of carbohydrate had a direct stimulating effect on insulin production and that the hypoglycemic phase of the ordinary tolerance curve is due to its overproduction. Thus a low carbohydrate diet means less need for insulin with resultant decrease in its production. If, at this point, large amounts of carbohydrates are suddenly fed, however, enough insulin is not immediately produced to take care of it. Most authors feel that marked hyperglycemia depends on decreased glycogen deposition in the liver as a consequence of insulin deficiency. It is well to remember that in diseases of the liver carbohydrate assimilation is lowered and that there is the possibility of a low carbohydrate diet injuring liver cells. But whatever may eventuate in the realm of theory the practical demonstration of the influence of various diets on the blood sugar tolerance curve is of the utmost importance from a diagnostic point of view. The lesson to be learned is this; see that the patient is on a normal mixed diet for at least ten days or two weeks previous to the tolerance test, see also, that the food has actually been eaten. A careful checking of this source of error will do much to aid reliable interpretation of results.

THE EFFECT OF HYDRATION AND DEHYDRATION ON THE CURVE

Sweeney,[49] showed that hydrated animals exhibited a thirty minute delay in their postprandial blood sugar rise, the interpretation being that of a transient hydremia.

THE EFFECT OF EMOTION AND PAIN

Cannon et al[7] showed that cats, excited by the sight and sound of dogs for as short a time as one-half an hour, exhibited glycosuria, whereas after adrenalectomy, when exposed to the dog three times as long, they failed to exhibit it. Folin and Berglund,[18] fully substantiated emotional glycosuria on human beings.

More recently, however, Foster,[23] and Malmros[41] covering the same ground, came to the conclusion that there is no such definite effect.

THE EFFECT OF AGE

Various investigators have shown that the rise in the curve of individuals between fifty and seventy years of age is more marked and of longer duration than in younger people. Malmros,[41] found peaks up to 0.24 per cent as normal in such people.

THE EFFECT OF EXERCISE

Figures 14 and 15 are from Staub.[47] In figure 14 the fine line represents the curve obtained while the person is at rest, whereas

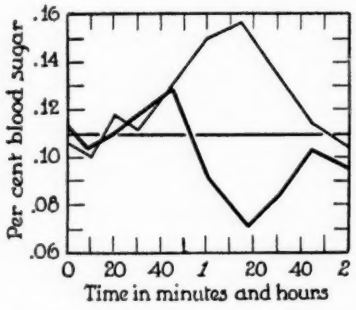

FIG. 14. REDRAWN FROM STAUB

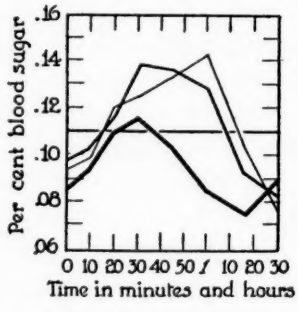

FIG. 15. REDRAWN FROM STAUB

the heavy line shows the effect of hard work for one hundred minutes immediately following ingestion of 20 grams of glucose. In figure 15, the fine line again representing the resting value, the moderately heavy line shows conditions after moderate exercise whereas the heaviest line shows the curve after heavy work. Hoffmann[32] said that both the positive and negative peaks of the curve disappear during work in a well trained person, while Staub explained the hypoglycemic peak during work on the basis that while at heavy work the ingested sugar is immediately used and added glucose is brought in to compensate.

EFFECT OF DRUGS

Blotner and Murphy[6] felt that liver contains a blood sugar reducing substance active and nontoxic when taken by mouth, hav-

ing an effect on blood sugar concentration similar to that obtained by insulin. Koplowitz[38] found that in giving 0.4 gram creatin by mouth the blood sugar is above 0.200 per cent and there is some effect on the curve, especially after insulin. Cori's[8] theory on hyperglycemia following epinephrine is well known. There is an irregular effect on the tolerance curve following thyroxin, salyrgan, foreign protein injection and possibly following salicylates.

THE EFFECT OF DISEASE

According to Joslin,[37] v. Noorden and Isaac,[53] hepatic and pituitary disease, pregnancy, asthma, certain cases of arteriosclerosis, certain types of organic and functional nervous disease, hyperthyroidism, obesity, chronic arthritis and certain types of carcinoma may produce a pathological alimentary hyperglycemia. Cardiac edema (Taterka et al[50]), shows a low fasting sugar and a slower rise and fall of the curve than normal, while in eclampsia there is a marked hypoglycemia just before the attack.

HEREDITY

This factor has not been sufficiently recognized as influencing carbohydrate metabolism. A very careful study on this subject was contributed by Hjarne,[31] and the following quotation of his summary seems to have been borne out by the other available data. His term orthoglycemic glycosuria means renal glycosuria. The important fact to remember is that in so far as is known, no transition occurs between this condition and true diabetes mellitus. A quotation from Hjarne runs as follows:

> Among 199 persons, interrelated by blood, the author has found:
>
> | | |
> |---|---|
> | orthoglycemic glycosuria in | 18 |
> | glycosuria on glucose test in | 6 |
> | glycosuria without diabetic symptoms in | 13 |
> | diabetes mellitus in | 7 |
> | transition case | 1 |
> | glycosuria with BLS-curve of definite type in | 1 |
>
> . . . . . .
>
> The cases of orthoglycemic glycosuria and glycosuria on glucose test and glycosuria without diabetic symptoms have been shown to be characterised by their clinical benignity and their non-development into diabetes mellitus.

These forms of glycosuria have been shown to be inheritable. They are inherited as monofactorial dominant characters.

Orthoglycemic glycosuria due to a lowered threshold, is to be regarded as a separate complaint.

No transition occurs between orthoglycemic glycosuria and diabetes mellitus. Diabetes mellitus has no effect on the hereditary transmission of orthoglycemic glycosuria. In marriage of persons who are probably both subjects of orthoglycemic glycosuria no summation occurs in the direction towards diabetes; diabetes does not arise.

Orthoglycemic glycosuria and diabetes mellitus have probably a different origin. Their occurrence in the same families may be a chance coincidence due

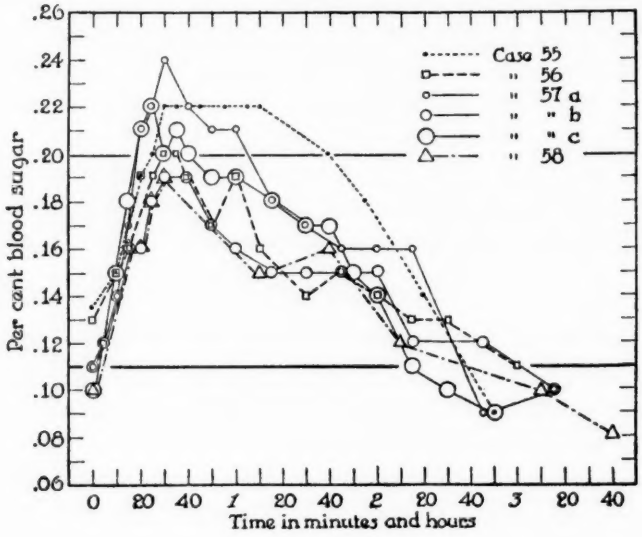

FIG. 16

to the fact that so far, it is mostly families with diabetes that have been subjected to careful search for glycosuria.

Figure 16 illustrates cases 55 to 58, inclusive, of Malmros,[41] series comprising six sisters and one brother all having glycosuria without subjective symptoms. He terms these "glycosuria innocens or diabetes mellitus?" There follows the quotation of his discussion,

Fasting blood sugar values of 0.13% were found in 3 of the patients (cases Nos. 55, 56, 57). [The figure shows lower fasting sugars for case 57, but his

statement refers to a sugar obtained previously—Author.] In 2 of them (cases Nos. 58 and 60) the fasting blood sugar was normal on ordinary diet. Nos. 59 and 61 were not more closely examined. None of the patients have had any subjective diabetic symptoms. Almén was pos. in 3 cases (Nos. 55, 56, 58) even on an empty stomach.

In case No. 55 a strong blood sugar rise (up to 0.22%) was found on the glucose tolerance test, after 2 hours the blood sugar was still high (0.18%). As the patient has a too high fasting blood sugar value and the blood sugar curve on the tolerance test mostly resembles a diabetes curve, the diagnosis diabetes mellitus can appear certain. There is, however, a possibility that a technical error can have been made on the blood sugar examinations, but the method has been controlled, as in all the other glucose tolerance tests, by control analysis on glycose solution of known concentration.

In case 56 the fasting blood sugar was also too high. On the glucose tolerance test a blood sugar rise up to 0.20% was found, 2 hours later the blood sugar was still too high (0.14%). This examination was made on the same day as the examination of case no. 55 but by another examiner. The method was also controlled here. Both these patients had kept diet, but the last few days before the examination they had been on ordinary mixed diet (but case No. 56 had not taken either sweet or sugar). One could possibly surmise that the patients became hypersensitive for carbohydrate by the preceding dietary treatment and that the sudden change of diet could be the cause of the increased fasting blood sugar value and the marked hyperglycemia on the glucose tolerance test. This does not appear very likely in the case of No. 55 as the preceding restriction of diet was only very moderate. Fasting blood sugar of 0.13% was established on two different occasions in case No. 57. On later examinations, however, the blood sugar was normal. In this case the glucose tolerance test was made on three different occasions. On two occasions the blood sugar rise was stronger than normal. The hyperglycemia was of longer duration than normal in all three examinations. One year elapsed between the second and third glucose tolerance tests. During this time the patient had kept ordinary mixed diet but consumed no sugar or sweets.

In cases Nos. 58 and 60 mainly normal blood sugar curve was found on the glucose tolerance test, but the hyperglycemia lasted (case No. 58) somewhat longer than normally.

The observation time for case No. 56 is 18 years, No. 57 6 years, No. 58 8 years, No. 60 8 years. Thus the glycosuria in these cases may be of a relatively benign nature. The question then arises:—Are cases Nos. 55, 56 and 57 to be regarded as diabetes or not? Case No. 58 ought to be designated as a glycosuria innocens with low threshold value ("renal" glycosuria). In cases Nos. 55 and 56 also the threshold value may be low (pos. Almén on an empty stomach). There could thus be found in the same family partly diabetes mellitus and partly benign glycosuria with a low threshold value. It could even be said that cases Nos.

55 and 56 are an example that there can be a low threshold value in diabetes mellitus. If no consideration is taken of the blood sugar examinations but only to the mild course, it is almost tempting to classify all these cases as "benign glycosuria." Also it hardly seems possible that it is a question of 2 completely different diseases, which appear in this manner in the same family. At present this question cannot be decided. The continued course will furnish an answer to this. The cases are of exceedingly great interest, both practically and theoretically. It is just such cases as these that can answer the question whether glyco-

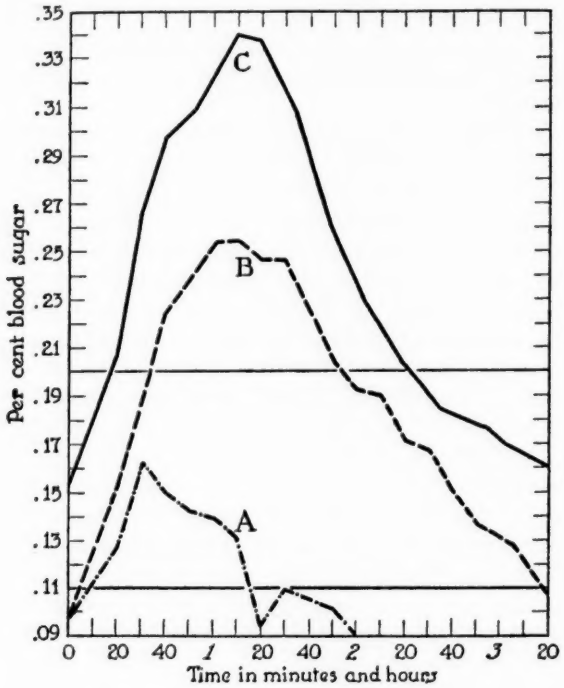

FIG. 17. REDRAWN FROM HATLEHOL

suria innocens with strong alimentary blood sugar rise is of a different nature than diabetes.

Figure 17 is taken from Hatlehol[30] case no. 53. A is the curve taken from the youngest daughter with cyclic renal glycosuria, B is the curve of the elder daughter who was a transition case and C is the curve of the father, who had benign diabetes. Under transition case is to be understood the type described by

Aage Jacobsen which is a glycosuria showing a great rise on administration of carbohydrates together with a low threshold. This, of course, corresponds with Holst's group IV shown in figure 8 under section D. Hatlehol, in common with others, found no progression into true diabetes in the transition cases.

Reference to figure 16 makes it obvious that in spite of the most accurate and refined methods there are still diagnostic problems. Two such will be illustrated from Malmros's series. Figure 18

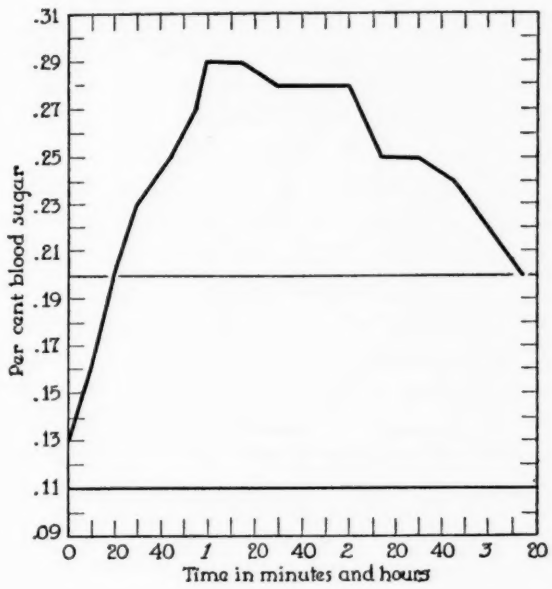

FIG. 18

shows the curve of case 62 in which the patient was under treatment for sciatica and whose history disclosed the fact that the father, mother and uncle had diabetes. Glycosuria was found and the first two blood sugar tests after fasting proved to be normal. From February 22 to March 9, on an ordinary mixed diet the sugars after fasting were not more than 0.11 per cent yet, as the figure shows, the tolerance test disclosed an abnormally high fasting sugar as well as a pathological curve throughout.

This case shows clearly that one cannot depend for diagnosis on a few normal blood sugar determinations after fasting. Figure 19 illustrates Malmros' case 64. There were no symptoms and the blood sugars after fasting were normal on ordinary mixed diet except on two occasions when they showed values of 0.14 and 0.13 per cent. The heavy line shows the first tolerance curve in which the fasting level is normal but the rise and duration are abnormal. The fine line shows the tolerance curve seven months later, which is entirely normal. The patient was on ordinary mixed diet dur-

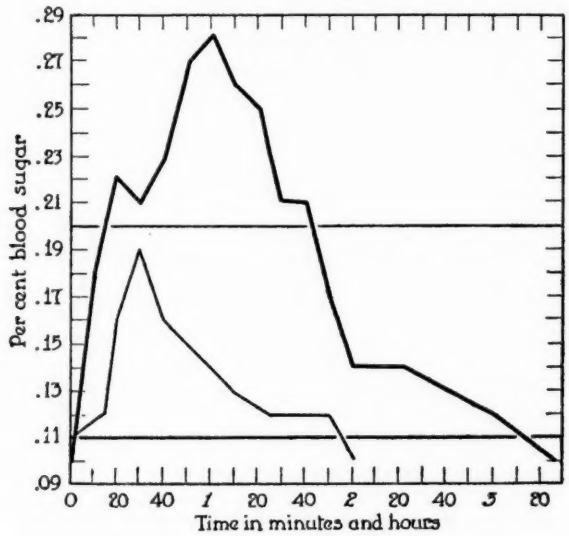

FIG. 19

ing the seven-month interval. To complicate matters still further, it was found that on the day following the second tolerance curve the fasting sugar was 0.13 per cent. Is this a case of diabetes or is it not?

DISCUSSION

It may be well to enquire briefly into the modifying factors that determine the shape of the blood sugar curve after the ingestion of glucose. There is the factor of rapid removal of sugar from the peripheral circulation during the period of absorption. Since the

early work of Claude Bernard the glycogen storing capacity of the liver has perhaps been foremost in our consideration and, therefore, the demonstration of a difference in sugar content between arterial and venous blood at the peak of the curve was at once unexpected and important. Among other things it partly explained the higher sugar readings obtained by Bang's method in contrast to the readings obtained by venipuncture. Folin, Trimble and Newman demonstrated that this rapid sugar removal from the periphery has less to do with glycogen formation in the muscles than with temporary storage without chemical alteration, particularly in the skin and subcutaneous tissue. This storing capacity is obviously diminished in diabetes, indeed in severe cases almost to a vanishing point. This might mean that the skin in diabetes carries a higher sugar concentration than in normal individuals, which lends new interest to the pathogenesis of various well known skin manifestations in this disease.

Physical removal of sugar from the circulation is not, however, the only factor at play, probably not even during the first minutes of the experiment, and in normal individuals it rapidly yields in importance to chemical removal, glycogen formation, and probably in a lesser extent to direct oxidation as well as synthesis of fat. The well known high respiratory quotients obtained after the ingestion of levulose are an indication in favor of the last alternative. The marked hypoglycemia following the hyperglycemia in normal individuals was discussed by MacLean and Wesselow as well as by Folin and Berglund before the arrival of insulin, and in the light of later experience must be taken as proof of a well working mechanism for the chemical disposal of sugar. Part of this mechanism must be the preparation or liberation of insulin, and it can be demonstrated, for example by the use of Woodyatt's pump for continuous injection, that time is required to raise it to a maximal working capacity. Using plenty of time for the stepping up of the sugar dosage, Berglund and Trimble reached decidedly higher tolerance levels than had been reported by Woodyatt and his collaborators, or than could be obtained by rapidly increasing the amount of sugar injected a minute. Therefore, no other single finding has done more to strengthen, theoreti-

cally as well as practically, the value of the tolerance curve than that of Malmros when he found 40 per cent of the ingested sugar still unabsorbed at a point when the blood sugar curve had already returned to normal after approximately two hours. Because of it one may well say that the declining part of the curve has little or nothing to do with the end of the absorption period but constitues a test for the mechanism of chemical sugar removal, or in most instances a test for the capacity to liberate insulin. This view harmonizes with the prolonged and even continuously rising curve in diabetes and constitutes the guide for the clinical use of the tolerance test. Theory and practical experience together make the prompt return of the sugar curve towards normal the most significant feature of this test when it is used for the study of sugar tolerance.

Up to the recent present it was believed that the metabolism of carbohydrate, protein and fat took place relatively independently of each other, at least as far as rate was concerned. No other dependence was suspected than the one discussed in terms of sugar and ketone body formation from protein, and of complete or incomplete oxydation of fat as a result of the simultaneous oxydation of glucose. The first well controlled experiments leading beyond this position were probably the ones carried out by Wilder and Boothby on the diabetic patient, Bessie B. It will be recalled that these experiments showed a very marked diminution of glucose tolerance when the protein level of the diet was raised without any simultaneous change in the total available glucose. A further step in the same direction is afforded by the recent work of Malmros and Sweeney with its highly significant demonstration of variability in the normal individual's reaction to glucose as the result of the characteristic dietary preceding the tolerance test. Thus when on a high fat diet or on prolonged fast, the individual responds to the sudden intake of glucose exhibiting a diabetic curve, in sharp contrast to the normal curve which is obtained when the preceding diet is dominated by carbohydrates, as a freely chosen diet usually is. Surprising also is the length of time that is required, according to Malmros, for a maximal adjustment from one type of diet to another. No doubt these

recent experiments will stimulate new work of a confirming or modifying character, but already the clear realization of the facts as they stand calls for greater caution in the carrying out and interpreting of glucose tolerance tests.

SUMMARY

A summmary of the more important diagnostic criteria to be considered in using the glucose tolerance test follows:

1. Never determine the blood sugar after fasting or do a glucose tolerance test on any patient until that patient has been on an ordinary mixed diet, unrestricted as to carbohydrate intake, for some days or weeks.

2. Use a reliable and accurate arterial method, and within the first hour take samples preferably at ten, twenty and thirty minutes, since the peak of the rise for the most part occurs at these periods.

3. Remember that the normal fasting blood sugar, both venous and arterial, should not exceed 0.11 per cent by any method used, except in people more than forty years of age or in those having febrile diseases, anemia, cerebral hemorrhage, or hyperthyroidism.

4. Determine the tolerance curve by using 1 gram of glucose per kilogram of body weight after a twelve hour fast, giving the glucose by mouth in a 10 per cent solution.

5. Keep in mind the fact that under these conditions in arterial blood the normal tolerance curve should not rise higher than 0.20 per cent and that the curve should fall to 0.11 per cent or below at the end of two hours.

6. Threshold determinations should be made in any case where the curve shows any abnormality in the presence of glycosuria, so that the type of glycosuria may be tentatively classified.

7. Remember that a fasting blood sugar of more than 0.11 per cent on any occasion is almost undoubtedly indicative of diabetes.

8. Do not be satisfied with a single fasting sugar. In a doubtful case do repeated fasting sugars over a period of months.

9. All cases showing a normal fasting sugar with a pronounced hyperglycemic curve of long duration should be suspected of diabetes and put under observation.

10. In abnormal cases of doubtful origin use the fermentation or phenylhydrazin test in the determination of type for the excreted sugar.

In addition one must (*a*) take a careful family history for possible glycosuria; (*b*) eliminate diseases, other than diabetes, that may cause disturbance of carbohydrate metabolism; (*c*) remember that long periods of observations, even up to forty years, seem to show that there is no transition in the same individual from non-diabetic glycosuria in any of the five classes discussed in this review to true diabetes mellitus, and (*d*) in cases without glycosuria, tolerance curves higher than normal do not necessarily indicate diabetes.

The author wishes to express his appreciation to Dr. Hilding Berglund for his valuable critical comment.

REFERENCES

(1) Andresen, J. and Schmidt, A.: Zur Frage des Blutzuckerspiegels bei Infektionskrankheiten. Klin. Wchnschr., **6,** 213. 1927.

(2) Bang, I.: Der Blutzucker. Wiesbaden, J. F. Bergman, 1913, 162 pp.

(3) Bang, I.: Ein Verfahren zur Mikrobestimmung von Blutbestandteilan. Biochem. Ztschr., **49,** 19–39. 1913.

(4) Barrenscheen, H. K.: Über Glykogen-und Zuckerbildung in der isolierten Warmbluterleber. Biochem. Ztschr., **58,** 277–314. 1914.

(5) Benedict, S. R.: The determination of blood sugar. Jour. Biol. Chem., **64,** 207–213. 1925.

(6) Blotner, H. and Murphy, W. P.: Effect of certain liver extracts on the blood sugar of diabetic patients. Jour. Am. Med. Assn., **94,** 1811–1816. 1930.

(7) Cannon, W. B., Shohl, A. T., and Wright, W. S.: Emotional glycosuria. Jour. Physiol., **39,** 280, 1911.

(8) Cori, C. G.: Insulin and Epinephrine. The Harvey Lectures. Baltimore, The Williams & Wilkins Co., 76–114. 1927–28.

(9) Cori, C. F., Pucher, G. W., and Bowen, B. D.: Comparative Study of the Blood Sugar Concentration in the Arterial and Venous blood of diabetic patients during insulin action. Proc. Soc. Exper. Biol. & Med., **21,** 122. 1923–24.

(10) Dorle, M., and Frank, W.: Vergleichende Blutzucker Untersuchungen an Kapillarem und venosem Blute bei Gesunden, Hypertonikern und Luetikern. Biochem. Ztschr., **179,** 252–260. 1926.

(11) Dorle, M., and Liehr, W.: Vergleichende Blutzuckeruntersuchungen and Kapillarem und venosem Blut nach Muskelbewegung. Biochem. Ztschr., **85,** 365–372. 1927.

(12) Elias, H.: Über die Rolle der Saure im Kohlenhydratstoffwechsel. Über Saurediabetes. Biochem. Ztschr., **48,** 120–143. 1913.

(13) Faber, K.: Lectures on Internal Medicine, Hoeber. 1927, 147 pp.

(14) Faber, K.: Bestimmung der Blutzuckerschwelle bei der Glycosurie. Wien. med. Wchnschr., **73,** 1189–1192. 1923.

(15) Faber, K., and Hansen, K. M.: The determination of the Threshold of Glycosuria and the errors involved. Acta. Med. Scandinav., **58,** 372–395. 1923.

(16) Faber, K., and Norgaard, A.: Studies on the Threshold of Glycosuria. Acta. med. Scandinav., **54,** 289–322. 1920–21.

(17) Folin, O.: An improved method for the determination of Uric Acid in blood. Jour. Biol. Chem., **86,** 179–187. 1930.

(18) Folin, O., and Berglund, H.: Some new observations and interpretations with reference to transportation, retention and excretion of carbohydrates. Jour. Biochem., **51,** 213–273. 1922.

(19) Folin, O., and Svedberg, A.: Sugar in urine and in blood. Jour. Biol. Chem., **70,** 405–426. 1926.

(20) Forsgren, E., Holmgren, H., Wilander, O., and Agren, G.: The connection between the functional activities of the liver and the susceptibility of the organism to insulin. Acta med. Scandinav., **73,** 60–70. 1930.

(21) Foster, G. L.: Studies on Carbohydrate metabolism. Jour. Biochem., **55,** 291–314. 1923.

(22) Friedenson, M., Rosenbaum, M. K., Thalheimer, E. J., and Peters, J. P.: Cutaneous and Venous blood sugar curves. Arch. Int. Med., **43:** 633–652. 1929.

(23) Friedenson, M., Rosenbaum, M. K., Thalheimer, E. J. and Peters, J. P.: Cutaneous and venous blood sugar curves. I. In normal individuals after insulin and liver disease. Jour. Biochem., **80,** 269–288. 1928.

(24) Gray, H.: Blood sugar standards in normal and diabetic persons. Arch. Int. Med., **31,** 241–258. 1923.

(25) Hagedorn, H. C.: Undersøgelser Vedrørende Blodsukkerregulationen hos Mennesket med saerligt henblik paa de kroniske glycosuriers differential diagnose. København. 1921.

(26) Hagedorn, H. C., and Jensen, Norman B.: Om kvantitativ Bestemmelse af minimale Glukosemaengder saerlig i blod. Ugeskrift for Laeger., **80,** 1217–1918.

(27) Haldane, J. B. S.: Experimental and therapeutic alterations of human tissue alkalinity. Lancet., **206,** 537–538. 1924.

(28) HAMMAN, L., AND HIRSCHMAN, I. I.: Studies on blood sugar. Arch. Int. Med., Chicago **20,** 761–808. 1917.

(29) HANSEN, K. M.: Investigations on the blood sugar in man conditions of oscillations, rise and distribution. Acta med. Scandinav. Supp. **4,** 1–224. 1923.

(30) HATLEHOL, R.: Blood sugar studies. Acta med. Scandinav., Supp. **8–10,** 1–260. 1924–25.

(31) HJARNE, U.: A study of orthoglycemic glycosuria with particular reference to its hereditability. Acta. med. Scandinav., **67,** 423–571. 1927.

(32) HOFMANN, A.: Über den Einfluss des Trainings auf den Ablauf der Arbeitsblutzucker Kurve des Stoffwechselgesunden Menschen. Klin. Wchnschr., **7,** 2043–2045. 1928.

(33) HOLST, J. E.: Investigations into benign glycosuria and diabetes mellitus. Acta. med. Scandinav., **63,** 47–98. 1925–26.

(34) JACOBSEN, A. T.: Blodsukkerindholdet hos Normale og ved diabetes mellitus. Københ. 1917.

(35) JACOBSEN, A. T.: Untersuchungen über den Einfluss des chlorohydrats auf experimentelle Hyperglykämieformen. Biochem. Ztschr., **51,** 443–462. 1913.

(36) JOHNS, H. J.: Pitfalls in the diagnosis of diabetes. Amer. Jour. Med. Sci., **159,** 102–111. 1925.

(37) JOSLIN, E. P.: The treatment of diabetes mellitus. Philadelphia and New York. 1923, pp. 784.

(38) KOPLOWITZ, E.: Wechselbeiziehungen zwischen Kreatin und Kohlehydratstoffwechsel. Einfluss peroraler Kreatingaben auf Blutzuckerspiegel und Insulinwirkung. Ztschr. f. klin. Med., **112,** 150–164. 1929.

(39) LIEFMANN, E., AND STERN, R.: Über Glykaemie und Glykosuria. Biochem. Ztschr., **1,** 299–308. 1906.

(40) MACLEAN, H., AND DEWESSELOW, O. L. V.: The estimation of the sugar tolerance. Quart. Jour. Med., **14,** 103. 1921.

(41) MALMROS, H.: A study of glycosuria, with special reference to the interpretation of the incidental finding of a positive reduction test. Acta. med. Scandinav., Supp. **27,** 1–309. 1928.

(42) PICKARD, R. J., AND PIERCE, L. F.: Blood dextrose determinations. Jour. Amer. Med. Assn., **94,** 480–483. 1930.

(43) PUNSCHEL, A.: Der Blutzucker im höheren Lebensalter unter besenderer Berücksichtingung der alimentären Hyperglykämie. Ztschr. f. klin Med. **96,** 253–278. 1923.

(44) RABINOWITCH, I. M.: Simultaneous determinations of Arterial and Venous Blood sugars in diabetic individuals. Brit. Jour. Exper. Path., **8,** 76–84. 1927.

(45) RABINOWITCH, I. M.: Blood sugar time curves. Jour. Clin. Investigation, **2,** 579–586. 1926.

(46) SEYDERHELM, R., AND OESTREICH, C.: Das "Tagesprofil" des Blutzuckers beim Gesunden und beim Diabetiker. Ztschr. f. klin. Med., **109,** 35–40. 1929.

(47) STAUB, H.: Untersuchungen über den Zuckerstoffwechsel des Menschen. Ztschr. f. klin. Med., **93,** 123–140. 1922.

(48) STAUB, H.: Untersuchgen über den Zuckerstoffwechsel des Menschen. Ztschr. f. klin. Med., **91,** 44–60. 1921.

(49) SWEENEY, J. S.: Dietary factors that influence the dextrose tolerance Test. Arch. Int. Med., **40,** 818–830. 1927.

(50) TATERKA, H.: Oestreicher, F.: Zuckerstoffwechsel und Wasserhaushalt. Klin. Wchnschr., **8,** 1401–1402. 1929.

(51) TRAUGOTT, CARL: Die Unterscheidung von innocenten und diabetischen glykosurien durch eine neue Untersuchungsmethode. Ztschr f.d. ges. exper. Med., **31,** 282–302. 1923.

(52) TRAUGOTT, CARL: Über das Vershalten des Blutsuckerspiegels bei wilder holter und verschiedener Art enteraler Zuckerzufuhr und dessen Bedeutung fur die Leberfunktion. Klin. Wchnschr., **1,** 892–894. 1922.

(53) VON NOORDEN, C., AND ISAAC, S.: Die Zuckerkrankheit und ihre Behandlung. Berlin, 1927.

(54) WILLIAMS, J. R., AND HUMPHREYS, E. M.: Clinical significance of blood sugar in Nephritic and other diseases. Arch. Int. Med., **23,** 537–581 1919.

(55) WISLICKI, L.: Das Verhalten des Blutzuckers nach intravenöser Traubenzuckerinjektion. Deutsche med. Wchnschr., **54,** 1831–1832. 1928.

MORPHOLOGIC AND CULTURAL STUDY OF STAPHYLOCOCCI WITH SPECIAL REFERENCE TO SOURCE

LUTHER THOMPSON

Section on Clinical Pathology, The Mayo Clinic, Rochester, Minnesota

This study was made in an attempt to evaluate the various Gram-positive cocci which occur in cultures of blood particularly, and in clinical bacteriologic work generally. Finding and reporting a staphylococcus in a large majority of cases is not of value to the clinician, as there is no apparent connection with the history or clinical symptoms. There are exceptions to this rule, for example, staphylococcic bacteremia following local infections with staphylococci or the association of staphylococci with boils, carbuncles and osteomyelitis.

This suggests that there is a sharply defined and restricted group of organisms responsible for certain types of primary infectious processes, and also that there are many miscellaneous organisms which are often encountered and which must be considered extraneous. There seems to be no classification available which allows the clinical bacteriologist to separate one group from the other without the expenditure of considerable time. Even then doubts arise as to the relative importance of the various tests employed.

Considerable work has been done on the classification of the mass-forming Gram-positive cocci, and has resulted in the accumulation of a large amount of data. Few of these data are useful in clinical work on account of the time consuming procedures employed and the number of strains of cocci encountered which do not seem to fit into any existing classification. Winslow, Rothberg and Parsons[8] studied 180 strains of staphylococci, 104 of which were from pathologic material; the remainder were

isolated from the skin or from air, dust and water. These investigators proposed a classification based on production of pigment, fermentation of lactose, and liquefaction of gelatin. Six species of staphylococci were given.

Hine[2] studied ninety strains of staphylococci assembled without selection from the following sources: blood, boils, throats, sputum, lesions of osteomyelitis, urine and skin. These he divided into group 1 (pyogenic) which fermented mannite, and group 2 (epidermidis) which did not ferment mannite. A serologic study showed that there were three types in group 1 according to absorption of agglutins, and two types in group 2. Julianelle[6] concluded that hemolysis by staphylococci is related to proteolytic activity. The twenty-five strains studied by him were alike in fermentation of sugar. Certain irregularities were noted in the liquefaction of gelatin. By the absorption of agglutinin test there were three groups with two subgroups. Hudson[5] investigated the staphylococci of throats of normal persons and the throats of persons with colds. He found that mannite is often fermented by *Staphylococcus aureus*. Kligler and Kraus[7] worked with thirty-three strains of staphylococci isolated from such sources as furuncles, abscesses, mastitis, pyodermitis, osteomyelitis and eczema. Of the thirty-three strains, twenty-six were pigmented and seven were white; twenty-five of the twenty-six pigmented strains fermented lactose and mannite, and twenty-two liquefied gelatin. There was poor agreement as to cultural characters among the white strains. Agglutination tests indicated four groups.

The most extensive investigation of the staphylococci since the work of Winslow, Rothberg and Parsons, seem to have been made by Yoshioka[9] who studied 180 strains with regard to fermentation of sugar, action on milk and gelatin, production of hemolysin and staphylokinase, agglutination, and pathogenicity (mouse). The sources of the 180 strains were as follows: 127 from incised abscesses or in pure culture in open wounds; twenty from normal mucosa, skin and air, and thirty-three from sources such that pathogenicity could not be predicted. Using the 127 strains obtained from what he considered to be primary staphylococcic

infections as a criterion for judging the value of the various tests applied, Yoshioka found that fermentation of sugar was the most valuable means of separating the pathogenic from the non-pathogenic staphylococci. Galactose, mannose, trehalose and lactose were positive with the 127 strains, and arabinose, raffinose and salicin were negative. These sugars were considered the most useful for differentiation while others were not helpful. The author then compared other tests with fermentation of sugar, and found that production of hemolysin was almost as good, since it gave 150 positive reactions as compared to 152 reactions with sugars. Liquefaction of gelatin gave a higher number than fermentation of sugar whereas the other tests gave lower figures. The chief criticism to be made is that the finding of Gram-positive cocci in pure culture in an open wound is not proof that they are the primary cause of the wound. Nor is it logical to assume that cocci found in pure culture in postoperative infections are necessarily of the same type as those found in spontaneous infections.

Dudgeon and Simpson[1] collected fifty strains of *Staphylococcus aureus* and eighty strains of *Staphylococcus albus* from "acute and chronic infections in man." All of the strains of *Staphylococcus aureus* liquefied gelatin and fermented lactose, and forty-seven fermented mannite. The strains of *Staphylococcus albus* gave inconsistent results. Hemolysis was produced on blood agar by 68 per cent of the strains of *Staphylococcus aureus* and 56 per cent of the strains of *Staphylococcus albus*. The precipitin reaction did not serve to separate the yellow and white cocci. Hucker and Rettger[4] pointed out that ammonium salts may serve as the only source of nitrogen for the saprophytic micrococci when used in a medium of definite chemical composition, whereas the parasitic forms fail to grow in such a medium. Hucker[3] presented a key for the identification of cocci included in Bergey's Manual under the genera Gaffkya, Staphylococcus and Micrococcus. He listed nineteen species and made the primary separation on production of pigment. Other characters were reduction of nitrates, liquefaction of gelatin and utilization of monobasic ammonium phosphate as a source of nitrogen.

PRESENT INVESTIGATION

In the present work 150 strains of cocci of the staphylococcus-micrococcus group were studied. They were divided into three groups of fifty strains each, according to source, as follows: group 1, scrapings from normal skin, group 2, cultures of blood in cases in which only an occasional colony, or at most a very few colonies, appeared on the plates, or in which growth appeared in broth but not on plates, and group 3, material from pyogenic infections. Exceptions to the foregoing are that, in group 2 one culture was included which was obtained from a typical staphylococcic bacteremia, and in group 3, five strains were included which were of doubtful significance, as well as could be judged from the original cultures. Of these five strains, three were from cultures of blood, one was from a culture from a prostate gland, and one was from culture of spinal fluid. The 150 strains were tested for ability to ferment lactose, saccharose, mannite and raffinose, to liquefy gelatin, to produce hemolysis on blood-agar plates and to produce pigment. The morphology was studied by preparing gram stains from nutrient agar slant cultures twenty-four hours old. The first twenty strains of each group were tested for ability to grow on a synthetic medium with ammonium oxalate as the source of nitrogen. Positive growths were not observed, and it was therefore concluded that organisms from the sources chosen do not often fall within the saprophytic group mentioned by Hucker and Rettger. The strains in group 3 were from boils in eighteen instances, from osteomyelitis in five, from wounds in eleven, from blood in six (four cases) from infected finger in three, from infected foot in two; from empyema fluid in two, from duodenal drainage in one instance; from the prostate gland in one; and from the spinal fluid in one.

It will be seen from table 1 that groups 1 and 2 are comparable, and that any one sugar will not serve to distinguish true staphylococci from saprophytic cocci. Mannite is more useful than any of the other sugars used. In order to get a fairly accurate cultural differentiation, lactose, mannite and gelatin may be used, and a test made for hemolysis. Most pyogenic staphylococci ferment lactose and mannite, liquefy gelatin, and

produce hemolysis when streaked on blood-agar plates. Six strains from group 3 which should be typical pyogenic staphylococci according to source failed in some one of these tests, yet morphologically they were typical of the pyogenic group. In this same group forty-five strains produced yellow pigment, whereas, five were not pigmented. One of the white strains which was obtained from a boil gave all the cultural and morphologic characters of the pyogenic type, and one yellow strain which was obtained from a culture of blood, did not correspond either culturally or morphologically with the pyogenic type. The latter case was one of infection of the foot following a bruise

TABLE 1

SUMMARY OF RESULTS OBTAINED IN THE VARIOUS GROUPS

	SOURCE	CULTURES	POSITIVE FOR LACTOSE	POSITIVE FOR SACCHAROSE	POSITIVE FOR MANNITE	POSITIVE FOR RAFFINOSE	POSITIVE FOR GELATIN	POSITIVE FOR HEMOLYSIS	TYPICAL MORPHOLOGY	YELLOW PIGMENT
Group 1.....	Skin	50	39	46	13	2	20	12	4	5
Group 2.....	Atypical cultures of blood	50	41	49	16	2	29	26	5*	7
Group 3.....	Pyogenic infection	50	44	50	44	0	44	45	45*	45
Selected from group 3.....	Boils and osteomyelitis	23	22	23	23	0	23	22	23	23

* Includes three cultures from one case.

and a typical staphylococcus was obtained from the wound. Thus it is seen that with two exceptions the strains in group 3 could be separated into typical staphylococci on one hand and micrococci on the other by production of pigment alone. The occasional failure of lactose to distinguish the pyogenic staphylococci from the micrococci is well illustrated in the following: Three cultures were obtained from the same case, one culture of blood antemortem, one culture of blood postmortem, and one culture taken from a furuncle. All three cultures failed to ferment lactose even after thirty days, but otherwise they were typical of the pyogenic group, culturally and morphologically. The case was one of diabetes and furunculosis.

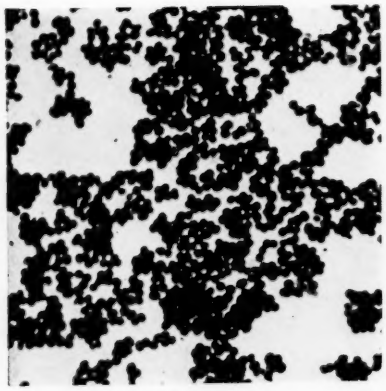

FIG. 1

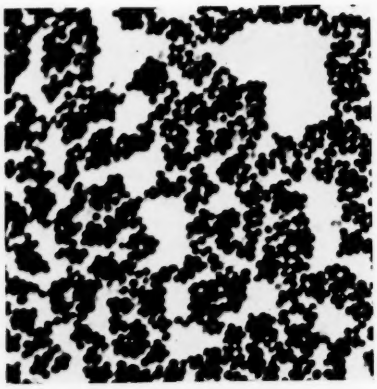

FIG. 2

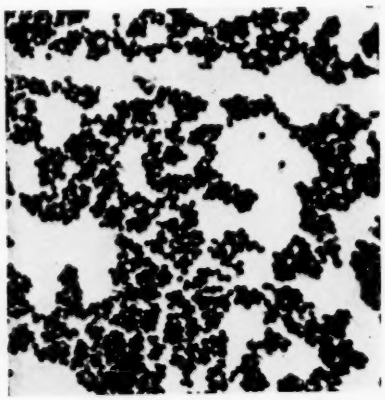

FIG. 3

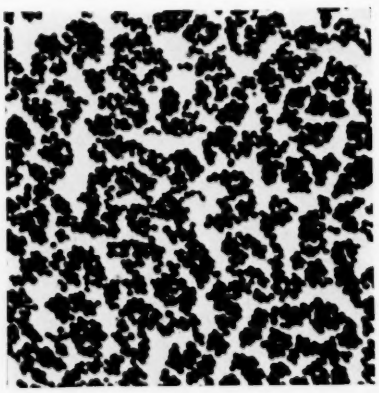

FIG. 4

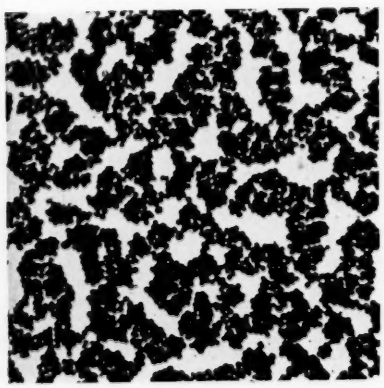

FIG. 5

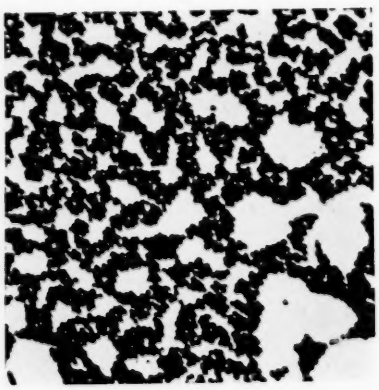

FIG. 6

Attention should be called to the similarity of the results obtained in groups 1 and 2. This suggests that the occasional colonies encountered in blood culture plates arose from organisms derived from the skin of patient or technician. In group 1 only four strains of typical staphylococci were found in a total of fifty, whereas in group 2, five strains of typical staphylococci were found in a total of fifty.

The greatest interest in this work is attached to the morphology. Little can be found in the literature or in textbooks

TABLE 2

GROUPING ACCORDING TO MORPHOLOGY OF MASS-FORMING COCCI ENCOUNTERED IN CLINICAL BACTERIOLOGY (PLATE)

FIGURE	SOURCE	GROUP	DESIGNATION	SIZE	LACTOSE	SACCHAROSE	MANNITE	RAFFINOSE	GELATIN	HEMOLYSIS
1	Skin	1	Micrococcus	1μ	+	+	−	−	−	−
2	Culture of the blood	1	Micrococcus	1μ	+	+	−	−	−	−
3	Skin	2	*Staphylococcus epidermidis*	0.7μ	+	+	−	−	+	−
4	Culture of the blood	2	*Staphylococcus epidermidis*	0.6–1.0μ	+	+	−	−	+	+
5	Osteomyelitis	3	Pyogenic staphylococcus	0.6μ	+	+	+	−	+	+
6	Furuncle	3	Pyogenic staphylococcus	0.6μ	+	+	+	−	+	+

which is helpful regarding the morphology of staphylococci and micrococci. Such descriptions do not mention the medium employed or the age of the culture, factors which are most important in determining the size and grouping of the cocci in question. The 150 cultures studied were all compared for morphology under as nearly similar conditions as possible. Gram stains were made from twenty-four hour cultures on nutrient agar slants having an initial reaction of pH 7.0. Care was taken to flame the slides before the smears were made so as to have the material spread evenly. Smears were made of such thickness

that fields might be observed which were well covered but not crowded so as to cause piling-up of the organisms unless they tended to do so naturally. The illustrations show what is considered about the right thickness of smears for easiest differentiation.

It was found that the 150 cultures could be divided into three types on the basis of morphology with the exception of two cultures from the skin which appeared to belong to the genus Sarcina. Figures 1 and 2 represent type 1 which comprises about 80 per cent of group 1, and 50 per cent of group 2. Most of the other organisms in these two groups fall into type 2, represented by figures 3 and 4. The typical pyogenic staphylococci in group 3 are represented by figures 5 and 6. Additional data concerning the strains represented by these figures are presented in table 2.

The organisms of group 1 were easily recognized by their greater size. The cocci appeared in clusters and tetrads. The organisms of group 2 were harder to distinguish from the typical staphylococci, which they more nearly approached in size. In general the organisms of group 2 were larger, less regularly round and varied more in size than those of the pyogenic type. The most helpful character in differentiation was the tendency to form clusters so that one organism lay above another on the slide. As opposed to groups 1 and 2, the cocci in group 3 were arranged in flat plates, almost all of them in one plane. Within these plates the cocci were so closely packed that they resembled the arrangement of cells in honeycomb. The outlines of individual cocci were distinct, owing to the distribution of the bacteria in one plane. These distinctions applied only to cultures examined under the conditions given, and not to smears made from broth cultures or from pus. A study of the morphology in hanging-drop cultures produced nothing helpful, chiefly on account of the difficulty of observing unstained bacteria.

COMMENT

On the basis of this study of 150 cultures it appears that most of the mass forming cocci encountered in clinical bacteriologic

work can be put into one of three groups. Those in group 1 appear to belong to the genus Micrococcus; those in group 2 correspond more nearly with the descriptions of *Staphylococcus epidermidis*. Both of these groups may well be looked on as incidental organisms in most cases, or as secondary invaders occasionally. Group 3 is essentially the pathogenic group. Some strains are white and correspond to descriptions of *Staphylococcus albus*, but most are pigmented and would be called *Staphylococcus aureus*. Most observers minimize the importance of production of the pigment as a differential character, since the pigment is variable under different conditions of culture, and since it may or may not be present even within the pyogenic group. The trinomial system formerly used designated these organisms as Staphylococcus pyogenes aureus, and Staphyloccus pyogenes albus. It would be more practical to combine both under the term *Staphylococcus pyogenes*. Strains fermenting raffinose were rarely encountered. Two were obtained from skin, two from cultures of blood, and none was obtained from typical staphylococcus infections. Three of these cultures resembled group 1 morphologically. The fourth resembled group 3 morphologically, it appeared as a single surface colony, on one of three plates used in making a culture of blood. Thus there is little evidence to justify a separate species within the pyogenic group corresponding to the descriptions of *Staphylococcus citreus*.

It is believed that a careful morphologic study of the mass-forming cocci obtained in clinical work will do much toward correlating the bacteriologic data with the clinical diagnosis. This can be done in twenty-four hours and has apparently given even more reliable results than the combined cultural tests including fermentation of sugars, and action on gelatin and on blood, which may require from one to three weeks.

SUMMARY

One hundred fifty strains of mass-forming cocci were studied culturally and morphologically as follows: fifty strains of cutaneous origin, fifty from atypical cultures of blood, and fifty from cases of pyogenic infection.

On morphologic grounds these 150 strains can be divided quite definitely into three groups, but less definitely by cultural procedures. The three groups are designated as Micrococcus, *Staphylococcus epidermidis* and *Staphylococcus pyogenes.*

It is believed that the morphologic study will offer a quick method of evaluating the various mass-forming cocci encountered in clinical bacteriology, and that it will greatly reduce the number of organisms reported as staphylococcus and increase the significance of those which are so reported, because of better correlation with clinical observations.

REFERENCES

(1) Dudgeon, L. S., and Simpson, J. W. H.: Differentiation of the staphylococci with special reference to the precipitin reaction. Jour. Hyg., **27:** 160–173. 1928.

(2) Hine, T. G. M.: Serological classification of the staphylococci. Lancet, **2:** 1380–1382. 1922.

(3) Hucker, G. J.: Further studies on the classification of the micrococci. Centralbl. f. Bakteriol., **111:** 9–22. 1929.

(4) Hucker, G. J., and Rettger, L. F.: The utilization of non-protein sources of nitrogen by the micrococci. Centralbl. f. Bakteriol., **65:** 273–277. 1925.

(5) Hudson, N. P.: The incidence and classification of staphylococci in the throats of normal persons and of persons with common colds; influenza studies. Jour. Infect. Dis., **32:** 297–306. 1923.

(6) Julianelle, L. A.: Studies of hemolytic staphylococci. Hemolytic activity—biochemical reactions—serologic reactions. Jour. Infect. Dis., **31:** 256–284. 1922.

(7) Kligler, I. J., and Krause, E.: The relationship of the orange and white pyogenic staphylococci, with special reference to vaccine therapy. Jour. Infect. Dis., **32:** 133–137. 1923.

(8) Winslow, C.-E. A., Rothberg, W., and Parsons, E. I.: Notes on the classification of the white and orange staphylococci. Jour. Bacteriol., **5,** 145. 1920.

(9) Yoshioka, Shinsaku: Classification of pathogenic and non-pathogenic staphylococcus by sugar splitting action. Japan Med. World, **4,** 32–35. 1924.

MODIFICATIONS OF DIFFERENTIAL STAINS WITH SPECIAL REFERENCE TO THE TRICHROMIC STAIN OF CAJAL

RAMON CASTROVIEJO

Suite A, 30 West 59th Street, New York City

Many pathologists use the silver impregnation methods of Cajal and his pupils for the investigation of the nervous system, but very few know and practice the triple stain, primarily described by Ramon y Cajal[3,4] in 1897, modified later by Calleja,[1] and Gallego.[2]

Without doubt this method is one of the best for staining all kinds of specimens composed of different structural elements, and especially for the study of tumors. Briefly the stain described by Cajal is as follow:

(1) Place sections for five or ten minutes in a saturated or very concentrated solution of basic fuchsin.

(2) Wash rapidly in water to remove the excess stain.

(3) Stain for five or ten minutes in a solution of picro-indigocarmin. (Saturated aqueous solution of picric acid, 100 cc. indigocarmin, 0.25 grams).

(4) Wash rapidly in dilute acetic-picric acid solution (3 drops of glacial acetic acid a few crystals of picric acid and 10 cc. distilled water).

(5) Pass the sections rapidly through water to remove the excess of picric acid and to avoid a green shade in the connective tissue.

(6) Decolorize in absolute alcohol until the sections have lost the excess of fuchsin, which will be known by the general violet color acquired by them. This step is the most delicate moment of the process and has to be performed in abundant alcohol in order to limit its duration to a few seconds. Leaving the sections too long in alcohol more or less decolorizes the nuclei.

(7) Clear in carbol-xylol, xylol and mount in balsam.

The nuclei appear intensely stained red, the cytoplasm takes a clear green or rose-yellow stain, and the connective tissue appears a very intense blue.

This method when properly carried out is without dispute one of the best differential stains, but it is difficult to execute because the alcohol acts as differentiating and dehydrating fluid at the same time. If the dehydration is prolonged too long the color of the nuclei disappears and the section appears with a uniform green-yellowish stain; if the sections are kept in alcohol too short a time the differentiation and dehydration do not take place, and the sections take a mixed purple color without any contrast.

To avoid the difficulty of keeping the stain in the nuclei Calleja[1] modified the technic using lithium carmin or borax carmin and counterstaining with picroindigocarmin. With this substitution the nuclei take a deep red color, and the path through the alcohol can be prolonged without fading the color of the nuclei. The differentiation is perfect, and the stain is permanent. Using this method Calleja has obtained beautiful slides of neoplasms.

I have been using this method for several years in sections of eyes embedded in celloidin, and I have obtained slides which demonstrate beautifully the histological and pathological structures of the eye.

Gallego introduced acetic acid as a differentiating fluid, and formaldehyd as a "viro-fixing" fluid for the sections stained with basic fuchsin, observing that after passing the sections previously stained in fuchsin through a solution of diluted formalin the stain was fixed in the nuclei and was insoluble in alcohol.

He[2] modified the trichromic methods of Cajal and van Gieson, and introduced basic fuchsin associated with eosin, to substitute for the ordinary hematoyhlin and eosin stain.

TRICHROMIC STAIN OF CAJAL MODIFIED BY GALLEGO

(1) Fix in 10 per cent formaldehyde (sections made by freezing microtome or embedded in paraffin or celloidin).

(2) Stain one minute in Ziehl's acetic-fuchsin, (Ziehl's fuchsin 10 drops, acetic acid 1 drop, distilled water 10 cc.

(3) Wash in water.

(4) Differentiation and "viro-fixation" is performed in formalin-acetic solution for five minutes (formalin 2 drops, glacial acetic acid 2 drops, distilled water 10 cc.).

(5) Wash in water.

(6) Picro-indigocarmin one minute (aqueous solution of indigocarmin 1 per cent, one part and aqueous saturated solution of picric acid, two parts).
(7) Wash in water, alcohol, carbol-xylol, et cetera.

The nuclei stain a deep red-violet; the cartilage, mucin and "Mazt-Zellen" in very intense bluish violet; the cytoplasm in clear green or yellowish green; the connective tissue an intense blue; the muscle fibers a clear green, et cetera.

This method has the advantage over the original Cajal method in that the nuclear stain is permanent after the sections are treated by formaldehyd, the differentiation is perfected by the addition of acetic acid to the fuchsin and formalin, and the alcohol completes the differentiation and dehydrates, but does not decolorize the nuclei.

VAN GIESON STAIN MODIFIED BY GALLEGO

The method of van Gieson is one of the most variable methods. Sometimes the nuclei take the stain very lightly, or appear with a red or brown color; at other times there is scarcely any noticeable differentiation between the connective tissue and the muscle fibers, and even the best sections fade in time.

The fundamental principle of the van Gieson stain and modifications of Weigert, Masson, Curtis and others, is the over-staining of the nuclei, and the staining and simultaneous differentiation with picro-fuchsin. In the method of Gallego the nuclear stain is weak, it is fixed with formalin, and the differentiation is very intense before the use of the picro-fuchsin. The technique follows:

(1) Fixation in 10 per cent formalin.
(2) Section by frozen, paraffin or celloidin methods.
(3) Acetic-fuchsin, one minute.
(4) Wash in water.
(5) "Viro-fixation" in acetic-formol, five minutes.
(6) Wash in water.
(7) Stain one minute with picro-fuchsin of van Gieson.
(8) Wash in water. Dehydration, carbol-xylol, xylol, et cetera.

The nuclei take a red-violet stain. The cartilage and mucin bluish-violet; the connective tissue intense red; the cytoplasm,

muscle fibers, red blood cells, yellow, bacteria red-violet. The stain is more constant in its results than the van Gieson, and is also more permanent.

BASIC FUCHSIN AND EOSIN STAIN OF GALLEGO

(1) Fix 10 per cent in formalin.
(2) Section by frozen paraffin or celloidin method.
(3) Stain one minute in acidified Ziehl's fuchsin, (fuchsin of Ziehl, 10 drops, glacial aceric acid 1 drop, distilled water 10 cc.).
(4) Wash in water.
(5) "Viro-fixation" in acetic-formalin.
(6) Wash in water.
(7) Counter stain with aqueous solution of one per cent eosin for one-half minute.
(8) Wash, alcohol, carbol-xylol, xylol, et cetera.

The aspect of the sections obtained by this method is similar to those obtained with hematoxylin and eosin. It has the advantage over the last method of more delicacy and beauty of the nuclear stain; the complete transparency of the sections is due to the double differentiation. The stain is almost specific for the fundamental substance of the cartilage, "Mazt-Zellen" of Ehrlich, some mucins, and bacteria are also stained.

For the study of elastic fibers I refer those interested to the work of Gallego.

AUTHOR'S MODIFICATION

I have been using the technic recommended by Gallego with a slight modification which simplifies the method. Ziehl's fuchsin in a solution of ten drops in 10 cc. of water is not sufficiently strong to stain all kinds of specimens, and many times one has to return the sections to the solution of fuchsin after their "viro-fixation" in formalin because of insufficient nuclear stain. To avoid this inconvenience the solution of fuchsin must be stronger, 15 to 20 drops for each 10 cc. of water, and added with two drops of formaldehyd, and 2 drops of acetic acid for each 10 cc. of water. With this solution of acetic-fuchsin-formalin, the stain, "viro-fixation" and differentiation takes place at the same time, and the degree of stain desired can be followed under the micro-

scope. The nuclear stain is completed in from one to five minutes, depending upon the specimens, and over-staining is impossible due to the presence of acetic acid in the solution.

This mixture of acetic-fuchsin-formalin changes color, gradually becoming darker and opaque, loses its selective properties and becomes useless in a few days. Inasmuch as the amount of fuchsin to be used daily is so small it is preferable to use fresh solution every day.

In order to continue the differentiation in this fluid, and to increase the selective properties of the stain which colors the young and old connective fibers in different grades of blue and green, a drop of acetic acid for each 10 cc. is added to the picro-indigocarmin solution. The details of the technic used by the author are as follows:

(1) Fix in 10 per cent formaldehyd.
(2) Section by frozen, paraffin or celloidin methods.
(3) Acetic-fuchsin-formalin (15 drops of fuchsin of Ziehl, 2 drops of glacial acetic acid, 2 drops of formalin, 10 cc. of water). Variable time as determined by observation.
(4) Wash in water.
(5) Indigocarmin-acetic (10 cc. of the solution of picro-indigocarmin of Cajal, 2 drops of glacial acetic acid).
(6) Wash rapidly in water.
(7) Alcohol, carbol-xylol, xylol, et cetera.

The color of the different structures is the same as in the Gallego stain. In most of the fresh specimens it will not be necessary to use the Ziehl fuchsin, since a 1 per cent solution of basic fuchsin will give the same results, but in older specimens the Ziehl fuchsin is more powerful for nuclear selection.

The first steps of my modification can be used combined with the picro-fuchsin of van Gieson or eosin as in the Gallego technic.

If some sections do not take the nuclear stain well they can be sensitized by keeping them for six to eight hours in a solution of formaldehyde at 45°C.

SUMMARY

For sections of the eyes embedded in celloidin, the method of Callaja will give excellent results.

The use of basic fuchsin associated with formalin and acetic acid and counterstained with picro-indigocarmin, picro-fuchsin or eosin, as recommended by Gallego or in my simplified technic is absolutely constant in its result, has the advantage over the original methods of Cajal and van Gieson of being more permanent, and easier to carry out.

The trichromic method of picro-indigocarmin and fuchsin is one of the best differential stains in use today, especially for the diagnosis of tumors, such as neurofibroma, myofibroma, myosarcoma, fibrosarcoma, et cetera and in tumors which have connective tissue framework associated with epithelial cells. In the squamous cell eqithelioma this method shows very beautifully the different degrees of keratinization and cellular differentiation with different tonalities of specific colors. It is also very useful for the study of blood vessels, demonstrating very well the various structures in pathologic arteries and veins, particularly in lesions of thrombo-angitis obliterans. This stain combines the advantages of hematoxylin and eosin staining and the van Gieson stain.

REFERENCES

(1) Calleja, C.: Método de triple coloración con el carmín litinado y el pirocarmín de índigo. Rev. trimest. microgr., **2,** 101–104. 1897.

(2) Gallego, A.: La fucsina básica y el formol en técnica histológica. Trab. de Labor. de Invest. biol. de la Univers. de Madrid, **17:** 95–110. 1919.

(3) Ramon y Cajal, S.: Manual de histológia normal y técnica micrographica. Valencia, 1897.

(4) Ramon y Cajal, S.: Manual de anatomía patológica general. 6th ed. Madrid, N. Maya, 1918. 616 pp.

EDITORIAL

The Relation of Calcium and Phosphorus Metabolism to Certain Non-Inflammatory, Non-Neoplastic Bone Diseases

The rôle of calcium in the production of bone is well recognized, but the fact that the bones act as a storehouse of calcium and other salts from which there is a continual ebb and flow received less attention up to the discovery by Collip of the active principle of the parathyroid glands (parathormone). Injection of this hormone may increase the blood calcium up to double the normal level and may increase the urinary excretion as much as ten times. Unless the ingestion of calcium is markedly increased during the administration of parathormone, the bone storehouse is depleted according to the work of Bauer, Aub and Albright, who demonstrated radiographically that the density of the bones was decreased. Hunter has shown histologically that the bone change takes place through a process of lacunar resorption by oseoclasts.

Although the most marked effects of parathormone are upon calcium metabolism, phosphorus excretion is also sharply increased and the blood phosphorus is decreased. Continued administration of the hormone has the reverse effect on the blood phosphorus.

Depletion of skeletal calcium in the form of generalized osteitis fibrosa was correlated with parathyroid disease through the accurate description of the disease by von Recklinhausen (1904), and the discovery in a typical case of a parathyroid tumor by Askanagy (1907); also through the demonstration of enlarged parathyroids in three cases of osteomalacia by Erdheim (1907), and the collection of twenty-seven cases of hypertrophy or adenoma of the parathyroids in seventeen cases of generalized osteitis fibrosa, eight cases of osteomalacia and two cases of

rickets by Hoffheinz (1925). The question as to whether the parathyroid changes were primary or secondary arose, but Schlagenhaufer and Maresch suggested that the parathyroids be removed in generalized osteitis fibrosa. Mandl followed this suggestion ten years later (1925), finding a tumor which was excised with marked improvement of the patient's condition. In the subsequent six years more than thirty-five cases of generalized osteitis fibrosa cystica have been operated. Both the experimental evidence and clinical experience with this disease demonstrate it to be one of calcium and phosphorus metabolism in which the parathyroids have a definite etiological significance in a majority of instances at least. Hunter, and Ballin and Morse suggest the name hyperparathyroidism or parathyroidism for this group of cases.

In contrast to generalized osteitis fibrosa the localized disease is not associated with appreciable changes in calcium and phosphorus metabolism.

Rickets and osteomalacia show normal blood calcium but a low inorganic blood phosphate. Pommer and Schmorl believe that the usual calcifying mechanism for conversion of osteoid tissue to bone is impaired. Although the mechanism is not understood, it is known that vitamin D supplies the deficiency and allows the normal utilization of calcium and phosphorus.

Hunger osteomalacia, so prevalent during the war, is considered a true osteomalacia by Schmorl and Partsch and is characterized by a marked osteoporosis. In some cases hypertrophy of the parathyroids is found and vitamin D is a specific.

The osteoporosis associated with hyperthyroidism is comparable anatomically with osteomalacia, especially of the hunger type, but chemically both the blood calcium and blood phosphates are normal. The phosphatase in the blood is increased and the excretion of both calcium and phosphorus is markedly elevated.

Renal rickets and the rare rickets associated with coeliac disease are interesting variants of this group. In renal rickets the phosphates are retained in the blood as they are in other types of renal sclerosis. The low blood calcium is considered to be secondary

to this retention of phosphates. As opposed to true rickets there is no vitamin D deficiency. Coeliac rickets simulates true rickets in that the negative calcium balance and associated osteoporosis may be combated with vitamin D.

Further study of the calcium and phosphorus metabolism must be made in not only the non inflammatory-non neoplastic diseases mentioned but also in Paget's disease, osteogenesis imperfecta, leontiasis ossea, fragilitas ossium and perhaps others, especially with reference to the parathyroids, thyroid, posterior lobe of the pituitary, the pancreas, and the kidneys since all of these organs are known to be capable of effecting the utilization, storage, retention, or excretion of either one or both the minerals in question.

F. W. HARTMAN.

NEWS AND NOTICES

The following committees have been appointed by the President:

Publication Committee

Dr. John A. Kolmer, *Chairman*
Dr. Kano Ikeda
Dr. William C. MacCarty

Editorial Policy Committee

Dr. T. B. Magath, *Chairman*
Dr. M. W. Lyon, *Assistant Chairman*
Dr. L. W. Larson
Dr. E. R. Mugrage
Dr. M. Pinson Neal

Committee on Award to International Men of Science Promoting Clinical Pathology

Dr. Walter E. King, *Chairman*
Dr. Albert H. Bradan
Dr. Stanley Reiman
Dr. Nathan Rosenthal
Dr. Henry C. Sweany

Committee on Publicity

Dr. C. I. Owen, *Chairman*
Dr. Philip Hillkowitz
Dr. C. W. Maynard
Dr. Herbert R. Mills

Committee on Honorary Membership

Dr. C. W. Maynard, *Chairman*
Dr. Charles R. Drake
Dr. Wm. M. Sheppe

Committee on Necropsies

Dr. O. A. Brines, *Chairman*
Dr. Israel Davidsohn
Dr. Rigney D'Aunoy
Dr. C. I. Owen
Dr. Ernest Scott
Dr. Walter Thomas

Committee on Exhibits to Medical and Hospital Associations

Dr. J. J. Moore, *Chairman*
Dr. Philip Hillkowitz
Dr. Oliver W. Lohr

Committee on Delegates to Medical and Hospital Functions

Dr. C. H. Manlove, *Chairman*
Dr. J. H. Black
Dr. W. G. Exton
Dr. Walter E. King

Round Table Committee

Dr. Mortimer Herzberg, *Chairman*
Dr. B. W. Rhamy
Dr. A. H. Schade

Committee on Scientific and Commercial Exhibits

Dr. Rigney D'Aunoy, *Chairman*
Dr. Willis P. Butler
Dr. Walter S. Thomas
Dr. Alfred S. Giordano

Announcement has just been made by the Wistar Institute of Anatomy and Biology that beginning in 1932 a new journal

will be published to be known as the Journal of Cellular and Comparative Physiology. The journal will be issued bimonthly and each volume will contain 500 pages. The Managing Editor is Dr. E. Newton Harvey of Princeton.

Dr. Louis B. Wilson was elected President of Sigma Xi National Honorary Society at the Convention held in New Orleans.

The following University appointments have been announced:

Dr. A. F. DeGroat has been appointed professor of Pathology at the University of Arkansas School of Medicine.

Dr. Stuart Mudd has been appointed associate professor of bacteriology at the University of Pennsylvania School of Medicine.

Dr. Sydney Dalrymple has been appointed instructor in pathology at Tufts College Medical School.

Dr. Louis Tuft has been appointed associate in immunology and chemotherapy at Temple University School of Medicine.

The School of Medicine at George Washington University has recently organized a faculty in contra-distinction to the teaching staff. Among the members are Dr. Earl B. McKinley, Bacteriology; Dr. Oscar B. Hunter, Pathology; Dr. Joseph H. Rowe, Biochemistry and Dr. George B. Roth, Pharmacology.

Dr. Esmond R. Long has recently been appointed director of laboratories of the Phipps Institute.

While all legislation pertaining to medicine is of interest to clinical pathologists, certain legislation is of special interest. The following items come under this category:

(1) The Seventy-second Congress, enacted Public Law No. 744, appropriating $75,000 for the purchase of a laboratory at Hamilton, Montana for the purpose of carrying on studies and research for the prevention, eradication and cure of Rocky Mountain spotted fever. A similar sum was appropriated for construction work. (2) Private law No. 299 approved and authorized a pension of $125 a month for James C. Burke, in compensation for

disabilities contracted by him when serving as a subject for experimentation during malarial fever investigations in the Philippine Islands.

The American Medical Association through its House of Delegates is attempting to have proper Federal Legislation enacted which will cure certain evils in the administration of the Veterans Bureau, in particular in regard to the treatment of veterans suffering from nonservice connected injuries. It is further attempting to guide Congress in regard to legislation pending which will prohibit the use of live dogs in the District of Columbia for purposes of research.

In surveying the bills which have passed state legislatures during the past year none seem to be of especial interest to clinical pathologists except as follows:

(1) Bills were killed in Colorado and Illinois which proposed to require that all pupils and teachers be immune to smallpox before they attend or teach school. An attempt to extend the provisions of the vaccination law to private schools was killed in Massachusetts. Bills were killed in Missouri which proposed that vaccination should not be made a condition precedent to the admission of any person into schools and colleges. Unsuccessful attempts were made in Connecticut and New York to amend the vaccination laws.

(2) Antivivisection bills were killed in Illinois, Maryland, New York and North Dakota.

(3) A North Carolina law limits the right to perform an autopsy on a dead human body to cases (1) in which an autopsy is specifically provided for by statute; or (2) was directed by the deceased; or (3) is deemed necessary on an inquest, by the coroner or a majority of a coroner's jury; or (4) is authorized by the husband or wife, or one of the next of kin or some other person charged by law with the duty of burial.

(4) A law enacted in Michigan provides for the registration and supervision of laboratories making chemical, serologic or bacteriologic laboratory tests to aid in the diagnosis or control of communicable diseases.

Again the attention of members is called to the Annual Con-

vention to be held in New Orleans on May 6–9. The headquarters is the Jung hotel and those expecting to attend are urged to send in their reservations immediately. Rooms may be held during the meeting of the American Medical Association. Those desiring to present papers and exhibits should send their titles to the Secretary not later than April 1.

Dr. Herzberg, Chairman of the round table committee, has announced that one of the subjects for discussion at the Convention will be the relation of the patient to the individual physician, the hospital staff and the hospital, as well as methods of remuneration. In this connection the following letter sent by Dr. Herbert Fox to a pathologist is of particular interest:

Dear Dr. ———

I have thought over your matter of the directorship of a hospital laboratory, and I have come to certain conclusions that I am sending to you and naturally would be willing to have known by anyone else who is interested in the matter.

I do not believe that a pathologist to a hospital, or indeed anyone occupying an academic position, should sign a contract. A pathologist is as much a chief and consultant as any other member of the staff, consequently equivalent to any staff chief. The pathologist should be in no way subordinate to a managerial director or the board of directors other than would be the case with a surgeon, pediatrist and the like.

His duties, if they have to be outlined and interpreted, are professional matters at the discretion of the staff. The amounts charged for private work within the hospital should be settled with the advice and agreement of the staff, but need not be a fixed schedule any more than any other professional compensation is. Any member of the staff should be willing to coöperate to the extent that he would be advised and influenced by the will of the staff, in so far as hospital matters are concerned.

The amount of money paid by the hospital to the pathologist depends upon the adoption of the above policy with the expression of opinion by the staff to the managers. The amount and methods seem to be open to respectable differences of opinion. It seems to me that one of two courses may be followed.

The pathologist might receive a respectable sum for all his time and service. All private fees from within or without the hospital should go to the hospital treasury. The amount paid to the pathologist could be settled in many ways, but it should equal the average professional income of the rest of the staff; this could be estimated by secret ballot.

The other method is that the hospital pay a moderate honorarium for the pathologist to supervise all the laboratory work. He should be permitted in addition to charge for private and semi-private work within the hospital and to accept external work. The details of executing this plan should be settled by a combined judgement of the staff and managers. Whatever the arrangements they should not infringe upon the professional independence of the pathologist.

The physical property of the laboratory should be supplied and maintained by the hospital and the pathologist should be of character and training that will care for it.

Finally, the position of a pathologist must remain a professionally ethical one upon a gentleman's agreement comparable to that holding good with his associates on the staff. If he would not carry out the spirit of such an agreement he could easily evade the letter of the contract. He is a member of the professional staff and as such cannot be treated as a subordinate by managerial officers.

This letter contains my own opinion and does not involve the University of Pennsylvania or any other institution with which I am connected.

BOOK REVIEWS

A Text Book of Laboratory Diagnosis. By EDWIN E. OSGOOD and HOWARD D. HASKINS. Pp. xix + 475, 1931, Philadelphia, P. Blakiston's Son & Company, $5.00.

This text is a compilation and development of the outline which has been used for some years in teaching laboratory diagnosis at the University of Oregon Medical School. It is therefore primarily developed as a text and while it will be useful as a laboratory manual it will be more valuable when used in a course of instruction. In order to make it more useful in this particular, the authors have included an index of diseases and an outline for laboratory class room work.

The subject matter is divided into two parts, the first of which deals with the knowledge which a practitioner should have available at the bed side of the patient, while the second part deals with laboratory methods. Since the authors have done their major research in hematology and blood chemistry, one is not surprised to find that these subjects are most thoroughly covered in the book. In connection with the chapters on hematology there are published six colored plates which are as good if not better than anything that has yet appeared in American text books. The authors have wisely given the colors which appear after Wright's staining method which is probably the most generally used in this country. The book does not deal adequately with the subject of bacteriology or serology which is evidently give as a separate course in the University of Oregon. For this reason the text is not a complete reference book in clinical pathology.

The sections dealing with animal parasites could stand some revision in order to clear up certain misstatements and to correct inaccuracies in the use of the specific names of parasites. For instance, while some have suggested that *Trichomonas vaginalis* and *Trichomonas hominis* are one and the same parasite, one is

certainly not justified in making the statement that chronic leucorrhea "should cause one to examine the vaginal secretion as well as the stool for the flagellate Trichomonas hominis." Further, the statement that nematodes have no intermediate hosts is obviously incorrect. The plate of animal parasites could also be revised to advantage and it would be especially desirable to state the magnification.

It would have been useful for the authors to have included the more simple Friedman test for pregnancy since it has come into so general use.

If one is justified in calling attention to these minor errors one should certainly call attention to the excellent presentation of the subject of clinical chemistry for here one can find an adequate treatment for all new phases necessary to this subject. All told the book makes an excellent addition to the clinical pathologist's armamentarium.

Approved Laboratory Technic. By JOHN A. KOLMER and FRED BOERNER. Pp. xxii + 663, 1931, New York and London, D. Appleton & Company, $7.50.

Although there are many books in the market dealing with laboratory technic, none so pretentious as this has ever appeared. The manual has been prepared under the supervision of the American Society of Clinical Pathologists and has been contributed to by various members of the Society. Each method has been submitted to various committees and groups of pathologists and therefore is in a sense a collection of "approved" methods. While one may differ with the authors as to which method should be used for a given test, nevertheless one will find in this manual adequate methods for performing almost any laboratory procedure. The manual is divided into the following sections: (1) General laboratory methods; (2) clinical pathology methods; (3) bacteriology methods; (4) serology methods; (5) clinical methods.

Even a casual glance of the chapter heads of this book will give one an idea of the enormous scope of the manual. One will not only find methods of doing almost any test necessary to perform

in a laboratory but one will also find information concerning the housing, feeding, inoculating, bleeding and autopsying of animals, methods for prevention and the emergency treating of laboratory accidents and one will also find methods dealing with milk examination, food examination, toxicological examinations and methods for the microscopic examination of tissues.

The book is profusely illustrated with excellent figures which have been beautifully reproduced. One might object to the fact that some of the color plates in hematology have been made after using Wilson's stain which is little used in this country and unfortunately the technic is omitted from the text. One may also object to the fact that the authors have not given the original source of some of the illustrations and some of the tests but in this day when literature is so profuse it is not a serious criticism.

Particularly valuable features of the book are the methods given for collecting material from patients and the exact method of procedure necessary to perform tests with good instructions for the use of the apparatus necessary and good figures.

All told the text is as near an encyclopedia of laboratory procedure as has yet appeared in this country and will not only be warmly received by clinical pathologists but will be frequently consulted by them as well as by technicians. The Society may well feel proud of having in a way had a hand in the development of this book.